Yellow pigment absent in male. Postdiscal and costal spots often black. Superficially similar to *glacialis* Butler.

### *Parnassius nordmanni* (Nordmann)

(Pl. 5, figs 35, 36)

*Doritis nordmanni* Nordmann, 1851 : 423, pl. 13, figs 1–3.
*Parnassius nordmanni* (Nordmann); Hemming, 1934 : 198.
*Parnassius nordmanni* Ménétriés; Bryk, 1935 : 146.
*Parnassius nordmanni* Ménétriés; Eisner, 1966 : 149.

DISTRIBUTION. U.S.S.R.: Caucasus.

### *Parnassius ariadne* Lederer

(Pl. 5, figs 37, 38)

*Doritis clarius* Eversmann, 1843 : 539, pl. 9, figs 1A, B, C (nec *clarius* Hübner, [1806] : 61, nota 6).
*Parnassius ariadne* Lederer, 1853 : 354.
*Parnassius ariadne* Lederer; Hemming, 1934 : 198.
*Parnassius clarius* (Eversmann); Bryk, 1935 : 151.
*Parnassius clarius* (Eversmann); Eisner, 1966 : 98.

DISTRIBUTION. U.S.S.R.: Russia (Altay), Tadzhikistan. Western Mongolia.

### *Parnassius clodius* Ménétriés

(Pl. 5, figs 39, 40)

*Parnassius clodius* Ménétriés, 1855 : 7.
*Parnassius clodius* Ménétriés; Bryk, 1935 : 156.
*Parnassius clodius* Ménétriés; Eisner, 1966 : 98.

DISTRIBUTION. U.S.A.: Alaska, Washington, Idaho, Montana, Wyoming, Oregon, Nevada. Canada: British Columbia.

LARVAL FOOD PLANTS. *Viola*, *Sedum* (Stonecrop), *Vaccinium?*, *Rubus?* (Wilson, 1961). *Saxifraga* sp., *Vitis californica* (Tietz, 1972).

### *Parnassius orleans* Oberthür

(Pl. 6, figs 41, 42, Text-fig. 27)

*Parnassius orleans* Oberthür, 1890 : 1.
*Parnassius orleans* Oberthür, 1891 : 8, 18, pl. 1, fig. 2.
*Parnassius orleans* Oberthür; Bryk, 1935 : 163.
*Parnassius orleans* Oberthür; Eisner, 1966 : 154.

DISTRIBUTION. Tibet. Mongolia. China: Sinkiang, Tsinghai, Kansu, Shensi, Szechwan, Yunnan.

## The *HARDWICKII*-Group

### ***Parnassius hardwickii*** Gray

(Pl. 6, figs 43, 44, Text-fig. 4)

*Parnassius hardwickii* Gray, 1831 : 32.
*Lingamius hardwickei* (Gray); Bryk, 1935 : 541.
*Lingamius hardwickei* (Gray); Eisner, 1966 : 120.

Distribution. Kashmir. N. India. Nepal. Sikkim. Bhutan.

Larval food plants. Various species of Saxifrage (Moore, 1902).

Superficially similar to *orleans* Oberthür but normally distinguishable by the white-centred, blue, submarginal series of spots on the hindwing upperside.

## The *SZECHENYII*-Group

### ***Parnassius szechenyii*** Frivaldszky

(Pl. 6, figs 45, 46, Pl. 15, fig. 100, Text-fig. 5)

*Parnassius szechenyii* Frivaldszky, 1886 : 39, pl. 4, figs 1, 1a.
*Koramius szechenyii* (Frivaldszky); Bryk, 1935 : 550.
*Koramius szechenyi* (Frivaldszky); Eisner, 1966 : 181.

Distribution. Tibet. China: Tsinghai, Kansu, Szechwan, Yunnan.

### ***Parnassius cephalus*** Grum-Grshimailo

(Pl. 6, figs 47, 48, Pl. 7, figs 49, 50, Text-fig. 22, 30)

*Parnassius cephalus* Grum-Grshimailo, 1891 : 446.
*Koramius cephalus* (Grum-Grshimailo); Bryk, 1935 : 558.
*Koramius cephalus* (Grum-Grshimailo); Eisner, 1966 : 97.

Distribution. Tibet. China: Kansu, Szechwan, Tsinghai. Kashmir.

Subsp. ***maharaja*** Avinoff [Kashmir: Ladakh Range]. Markings generally reduced. Submarginal spots above faint, not centred with blue. Postdiscal and costal spots absent from hindwing upperside.

This subspecies (Pl. 7, figs 49, 50), treated by both Bryk (1935) and Munroe (1960) as a species, differs from typical *cephalus* in having veins $R_1$ and $R_2$ consistently anastomosing, causing it to key out as *P. acco* Gray.

## The *ACCO*-Group

### ***Parnassius acco*** Gray

(Pl. 7, figs 51, 52, 53, 54)

*Parnassius acco* Gray, 1853 : 76, pl. 12, figs 5, 6.
*Tadumia acco* (Gray); Bryk, 1935 : 631.
*Tadumia acco* (Gray); Eisner, 1966 : 82.

DISTRIBUTION. Kashmir. Tibet. Sikkim.

Subsp. ***liliput*** Bryk [Tibet: Everest District], subsp. ***hunningtoni*** Avinoff [Tibet: Dochar, Tuna, Tsangpo Valley, Dzara, Kyetrak, Chumbi Valley. Sikkim: Gangtok]. Smaller than the typical form, red markings absent.

## THE *DELPHIUS*-GROUP

### ***Parnassius patricius*** Niepelt

(Pl. 7, figs 55, 56)

*Parnassius patricius* Niepelt, 1911 : 274.
*Koramius patricius* (Niepelt); Bryk, 1935 : 568.
*Koramius patricius* (Niepelt); Eisner, 1966 : 155.

DISTRIBUTION. U.S.S.R.: Kirghizia.

### ***Parnassius acdestis*** Grum-Grshimailo

(Pl. 8, figs 61, 62, Pl. 15, fig. 101, Text-fig. 7)

*Parnassius delphius* var. *acdestis* Grum-Grshimailo, 1891 : 446.
*Koramius acdestis* (Grum-Grshimailo); Bryk, 1935 : 572.
*Koramius acdestis* (Grum-Grshimailo); Eisner, 1966 : 82.

DISTRIBUTION. U.S.S.R.: Kirghizia. Kashmir. Tibet. Sikkim. Bhutan. China: Sinkiang, Szechwan.

The arrangement of the forewing radial veins is more variable in this species than in any other. Although veins $R_1$ and $R_2$ are usually separate, in many specimens they do appear to touch and in some cases quite definitely anastomose.

Subsp. ***lucifer*** Bryk [Sikkim: Gyamtshona]. Postdiscal and costal spots black in hindwing above.

Subsp. ***lux*** Eisner [Tibet: Jung-jung Khola]. Basal black scaling in hindwing above far less extensive than in the typical form. Postdiscal and costal spots large.

### ***Parnassius delphius*** (Eversmann)

(Pl. 8, figs 57, 58, 59, 60, Pl. 15, fig. 102)

*Doritis delphius* Eversmann, 1843 : 541, pl. 7, figs 1a, b.
*Parnassius delphius* (Eversmann); Elwes, 1886 : 39.
*Koramius delphius* (Eversmann); Bryk, 1935 : 583.
*Koramius delphius* (Eversmann); Eisner, 1966 : 102.

DISTRIBUTION. Afghanistan. U.S.S.R.: Tadzhikistan, Kirghizia, Uzbekistan. Pakistan. Kashmir. N. India. Tibet. China: Sinkiang, Tsinghai.

Highly variable species. Hindwing discal spots often without red scales.

Subsp. ***pulchra*** Eisner [Kirghizia: Kungey Alatau Mountains]. Wings exceptionally dark, semi-transparent.

Although treated by Bryk (1935) and Munroe (1960) as a distinct species, *Parnassius stoliczkanus* is here regarded, in accordance with Eisner (1966), as a subspecies of *Parnassius delphius* (Eversmann). The following subspecies are those that Bryk and Munroe would have included in *Parnassius stoliczkanus* Felder & Felder.

Subsp. ***atkinsoni*** Moore [Kashmir: Pir Pinjal, Sind Valley, Burzil Pass. India: Himachal Pradesh, Kulu], subsp. ***beate*** Eisner [Kashmir: Karakoram, Potu-la Pass, Chalsi, Leh], subsp. ***chitralica*** Verity [Pakistan: Chitral], subsp. ***florenciae*** Tytler [Tibet: Phupes Hundes, Tibu, Churmurti], subsp. ***gracilis*** Bryk & Eisner [India: Himachal Pradesh, Kangra, Rohtang Pass], subsp. ***imitator*** Bryk & Eisner [U.S.S.R.: Tadzhikistan, Pamirs, Beik Pass], subsp. ***kumaonensis*** Riley [India. Uttar Pradesh, Kumaon, Shillung], subsp. ***nicevillei*** Avinoff [Kashmir: Pir Pinjal, Burzil Pass, Sari Sungur Pass, Sapta La], subsp. ***parangensis*** Eisner [India: Himachal Pradesh, Parang Pass, Bara Lacha Pass. Kashmir: Tagalang Pass, Lingti, Ladahk], subsp. ***rileyi*** Tytler [Kashmir: Rupal Valley, Astor], subsp. ***spitiensis*** Bang-Haas [Tibet: Spiti, Tum-Tum-Thang, Churmurti], subsp. ***stoliczkanus*** Felder & Felder [Kashmir: Ladak, Rupshu, Sapta La], subsp. ***tenuis*** Bryk & Eisner [Kashmir: Gya-Ladahk, Tagalang Pass], subsp. ***tytlerianus*** Bryk & Eisner [Kashmir: Chitral, Bangol Pass], subsp. ***zanskarica*** Bang-Haas [Kashmir: Nira, Zanskar Mts.], subsp. ***zogilaica*** Tytler [Kashmir: Zogila]. Generally smaller than the typical *delphius*. Red costal spot of hindwing upperside usually absent.

## The *IMPERATOR*-Group

### ***Parnassius imperator*** Oberthür

(Pl. 8, figs 63, 64, Pl. 15, fig. 103, Text-fig. 21)

*Parnassius imperator* Oberthür, 1883 : 77.
*Tadumia imperator* (Oberthür); Bryk, 1935 : 675.
*Eukoramius imperator* (Oberthür); Eisner, 1966 : 123.

Distribution. Tibet. China: Tsinghai, Kansu, Szechwan, Yunnan.

Larval food plant. *Corydalis* (Verity, 1907).

## The *CHARLTONIUS*-Group

### ***Parnassius charltonius*** Gray

(Pl. 9, figs 65, 66, Pl. 15, fig. 104, Text-fig. 24)

*Parnassius charltonius* Gray, 1853 : 77, pl. 12, fig. 7.
*Koramius charltonius* (Gray); Bryk, 1935 : 694.
*Koramius charltonius* (Gray); Eisner, 1966 : 97.

Distribution. Afghanistan. U.S.S.R.: Kirghizia, Tadzhikistan. Pakistan. Kashmir. N. India. Tibet.

Larval food plant. *Corydalis gortschakovi* (A. Tsvetajev, pers. com.)

### ***Parnassius inopinatus*** Kotzsch

(Pl. 9, figs 67, 68, Pl. 15, fig. 105)

*Parnassius inopinatus* Kotzsch, 1940 : 17.
*Kailasius inopinatus* (Kotzsch); Eisner, 1966 : 123.

DISTRIBUTION. Afghanistan: Firus-Kuhi Range, Koh-i-Baba Range.

### ***Parnassius loxias*** Püngeler

(Pl. 9, figs 69, 70)

*Parnassius loxias* Püngeler, 1901 : 178, pl. 1, figs 5, 6.
*Koramius loxias* (Püngeler); Bryk, 1935 : 717.
*Eukoramius loxias* (Püngeler); Eisner, 1966 : 132.

DISTRIBUTION. U.S.S.R.: Kirghizia. China: Sinkiang.

### ***Parnassius autocrator*** Avinoff

(Pl. 9, figs 71, 72, Text-fig. 28)

*Parnassius charltonius autocrator* Avinoff, 1913 : 16, pl. 2, fig. 2.
*Koramius charltonius autocrator* (Avinoff); Bryk, 1935 : 716.
*Eukoramius autocrator* (Avinoff); Eisner, 1966 : 91.

DISTRIBUTION. Afghanistan. U.S.S.R.: Tadzhikistan.

LARVAL FOOD PLANT. *Corydalis adiantifolia* (Wyatt & Omoto, 1963).

## THE *TENEDIUS*-GROUP

### ***Parnassius tenedius*** Eversmann

(Pl. 10, figs 75, 76, Text-fig. 3)

*Parnassius tenedius* Eversmann, 1851 : 621.
*Tadumia tenedius* (Eversmann); Bryk, 1935 : 647.
*Tadumia tenedius* (Eversmann); Eisner, 1966 : 181.

DISTRIBUTION. Mongolia. U.S.S.R.: Russia (Yakut, Tuva, Chita). China: Inner Mongolia.

LARVAL FOOD PLANT. *Corydalis* sp. (*bracteata*?)( A. Tsvetajev, pers. com.).

## THE *SIMO*-GROUP

### ***Parnassius simo*** Gray

(Pl. 10, figs 73, 74, Text-fig. 8)

*Parnassius simo* Gray, 1853 : 76.
*Tadumia simo* (Gray); Bryk, 1935 : 654.
*Tadumia simo* (Gray); Eisner, 1966 : 178.

DISTRIBUTION. U.S.S.R.: Kirghizia, Tadzhikistan. Kashmir. N. India. Mongolia. Tibet. China: Sinkiang, Kansu.

## Tribe **ZERYNTHIINI**

Zerynthianae Grote, 1899 : 17. Type-genus: *Zerynthia* Ochsenheimer.

### *SERICINUS* Westwood

*Sericinus* Westwood, 1851 : 173. Type-species: *Papilio telamon* Donovan, by original designation.
*Sericinus* Westwood; Bryk, 1934 : 77.
*Sericinus* Westwood; Munroe, 1960 : 13.

### *Sericinus montela* Gray

(Pl. 10, figs 77, 78, Text-figs 16, 26)

*Papilio telamon* Donovan, 1798 : pl. 27, fig. 1. [Junior homonym of *Papilio telamon* Linnaeus, 1758 : 486.]
*Sericinus montela* Gray, 1853 : 78, pl. 13, figs 1, 2.
*Sericinus telamon* (Donovan); Bryk, 1934 : 80.
*Sericinus telamon montela* Gray; Bryk, 1934 : 89.
*Sericinus montela* Gray; Eisner, 1966 : 142.
*Sericinus telamon* (Donovan); Eisner, 1966 : 181.
*Sericinus montela* Gray; Hemming, 1967 : 409.

DISTRIBUTION. China: Heilungkiang, Kirin, Liaoning, Hopei, Shangtung, Anhwei, Kiangsu, Hunan, Hupeh, Kiangsi, Kansu. Korea.

LARVAL FOOD PLANTS. *Aristolochia* (Leech, 1893 : 488). *A. contorta* (Kurentsov, 1970).

### *PARNALIUS* Rafinesque

*Thais* Fabricius, 1807 : 283. Type-species: *Papilio hypsipyle* Fabricius, by monotypy. [Junior homonym of *Thais* Röding, 1789.]
*Parnalius* Rafinesque, 1815 : 128. [Replacement name for *Thais* Fabricius.]
*Zerynthia* Ochsenheimer, 1816 : 29. [Replacement name for *Thais* Fabricius.]
*Eugraphis* Billberg, 1820 : 75. Type-species: *Papilio hypsipyle* Fabricius, by monotypy.
*Parnalius* Rafinesque; Sherborn, 1929 : 4765.
*Zerynthia* Ochsenheimer; Sherborn, 1932 : 7041.
*Allancastria* Bryk, 1934 : 19, 61–62. Type-species: *Thais cerisy* Godart, by original designation. **Syn. n.**
*Zerynthia* Ochsenheimer; Bryk, 1934 : 31.
*Parnalius* Rafinesque; Neave, 1940*a* : 614.
*Zerynthia* Ochsenheimer; Neave, 1940*b* : 689.
*Allancastria* Bryk; Munroe, 1960 : 10.
*Zerynthia* Ochsenheimer; Munroe, 1960 : 13.
*Zerynthia* Ochsenheimer; Hemming, 1967 : 464.
*Parnalius* Rafinesque; Cowan, 1970 : 11. [*Zerynthia* Ochsenheimer cited as synonym.]

According to Cowan (1970) Rafinesque introduced the name *Parnalius* for *Thais* Fabricius, which was invalid as a junior homonym. The name is available and valid, and is a senior objective synonym of *Zerynthia* Ochsenheimer, 1816. It has been correctly listed by both Sherborn (1929) and Neave (1940).

The genus *Allancastria* Bryk is here treated as a synonym of *Parnalius* Rafinesque. The differences in venation, as figured by Bryk (1934), do not appear to be consistent although the genitalia are certainly distinct. If *Allancastria* Bryk is to be recognized as a valid genus it would seem to me that there is equal justification for raising the status of the species groups of *Parnassius* to genera. In order to maintain consistency in approach I am regarding *Allancastria* Bryk and *Parnalius* Rafinesque as being subjectively synonymous.

### Key to the species of *PARNALIUS* Rafinesque

1 Cell 2*A* of forewing underside uniformly scaled, without distinct red or black discal spot; uncus long, bifid; clasper narrow (Text-fig. 11, Pl. 10, figs 79, 80) ***cerisy*** (Godart) (p. 91)

– Cell 2*A* of forewing underside with a distinct red or black discal spot; uncus short, bifid; clasper broad (Text-figs 9, 10) . . . . . 2

2 (1) Forewing upperside usually with distinct red spots in the discal cell and with a vitreous spot near the wing apex; uncus in dorsal aspect narrowing towards the base; clasper distinctly produced dorso-posteriorly (Text-fig. 10, Pl. 11, figs 83, 84) . . . . . ***rumina*** (Linnaeus) (p. 92)

Forewing upperside usually with black spots in the discal cell, seldom red, and vitreous spot absent from the wing apex; uncus in dorsal aspect narrowing towards the apex; clasper slightly produced dorso-posteriorly (Text-fig. 9, Pl. 11, figs 81, 82) . . . ***polyxena*** (Denis & Schiffermüller) (p. 91)

## ***Parnalius cerisy*** (Godart) **comb. n.**

(Pl. 10, figs 79, 80, Text-figs 11, 17)

*Thais cerisy* Godart, [1824] : 812.
*Zerynthia cerisyi* (Godart); Stichel, 1907*a* : 82.
*Allancastria cerisyi* (Godart); Bryk, 1934 : 63.
*Allancastria cerisy* (Godart); Cowan, 1970 : 17, 41.

Distribution. Cyprus. Crete. Greece. Yugoslavia. Bulgaria. Rumania. Albania. Turkey. U.S.S.R.: Armenia. Iran. Iraq. Syria. Israel. Lebanon.

Larval food plants: *Aristolochia clematis* and *A. hastata* (Bryk, 1934). *A. maurorum* (Suguru Igarashi, in litt.).

## ***Parnalius polyxena*** (Denis & Schiffermüller) **comb. n.**

(Pl. 11, figs 81, 82, Text-figs 9, 19)

*Papilio hypermnestra* Scopoli, 1763 : 149, pl. [17], fig. 425. [Junior homonym of *Papilio hypermnestra* Linnaeus, 1763 : 40.]
*Papilio polyxena* Denis & Schiffermüller, 1775 : 162.

*Papilio hypsipyle* Fabricius, 1777 : 265.
*Zerynthia hypermnestra* (Scopoli); Bryk, 1934 : 34.
*Zerynthia hypermnestra* (Scopoli); Eisner, 1966 : 123.
*Zerynthia polyxena* (Denis & Schiffermüller); Hemming, 1967 : 436.

Hemming (1967) gives a detailed explanation of the nomenclatorial history of this species and how *polyxena* was finally established as the valid name.

DISTRIBUTION. S. France. Austria. Italy. Sicily. Yugoslavia. Hungary. Rumania. Albania. Greece. Czechoslovakia.

LARVAL FOOD PLANTS. *Aristolochia pistolochia*. *A. rotunda* and *A. clematis* (Higgins & Riley, 1970). *A. sicula* (Bryk, 1934).

### ***Parnalius rumina*** (Linnaeus) **comb. n.**

(Pl. 11, figs 83, 84, Text-fig. 10)

*Papilio rumina* Linnaeus, 1758 : 480.
*Thais maturna* Butler, 1870 : 232.
*Zerynthia rumina* (Linnaeus); Bryk, 1934 : 50.

DISTRIBUTION. S. France. Spain. Portugal. Algeria. Morocco. Tunisia.

LARVAL FOOD PLANTS. Various kinds of *Aristolochia* (Higgins & Riley, 1970). *A. pistolochia* and *A. fontanesi* (Bryk, 1934).

## ***LUEHDORFIA*** Crüger

*Luehdorfia* Crüger, 1878 : 128. Type-species: *Luehdorfia eximia* Crüger, by monotypy.
*Luehdorfia* Crüger; Bryk, 1934 : 99.
*Luehdorfia* Crüger; Munroe, 1960 : 13.

### KEY TO THE SPECIES OF *LUEHDORFIA* CRÜGER

1 Claspers of male covered with thick golden brown pubescence; ventral spinose setae, placed internally on claspers, elongate (Text-fig. 12); sphragis of female keeled (Pl. 11, figs 85, 86) . . . . . ***puziloi*** (Erschoff) (p. 92)
– Claspers of male covered with thick black pubescence; ventral setae, placed internally on claspers, shorter (Text-fig. 13); sphragis of female without keel (Pl. 11, figs 87, 88, 89, 90) . . . . . ***japonica*** Leech (p. 93)

### ***Luehdorfia puziloi*** (Erschoff)

(Pl. 11, figs 85, 86, Text-fig. 12)

*Thais puziloi* Erschoff, 1872 : 315.
*Luehdorfia eximia* Crüger, 1878 : 128.
*Luehdorfia puziloi* (Erschoff); Bryk, 1934 : 102.

DISTRIBUTION. U.S.S.R.: Russia (Primorye). Korea. Japan: Hokkaido, Honshu.

LARVAL FOOD PLANTS. *Asarum* (Graeser, 1888). *A. sieboldi* (Kurentsov, 1970).

### *Luehdorfia japonica* Leech

(Pl. 11, figs 87, 88, 89, 90, Text-figs 13, 23)

*Luehdorfia japonica* Leech, 1889 : 25, pl. 1, figs 1, 1b, 1c.
*Luehdorfia japonica japonica* Leech; Bryk, 1934 : 102.

DISTRIBUTION. Japan: Honshu. Taiwan. China: Liaoning, Kirin, Hupeh, Anhwei, Kiangsu, Kiangsi.

LARVAL FOOD PLANTS. *Asarum nipponicum, A. tamaense, A. blumei, A. caulescens* and *A. sieboldi* (Suguru Igarashi, in litt.).

Subsp. ***chinensis*** Leech (China: Hupeh, Anhwei, Kiangsu, Kiangsi). Hindwing upperside with red submarginal band. Internervular marginal spots of hindwing yellow.

The latter taxon, variously treated by authors, was regarded by Bryk (1934) as a subspecies of *puziloi* Erschoff. The male genitalia, however, show a close resemblance to those of *japonica* Leech both in the shape of the claspers and in the length of the setae placed internally thereon. These characters, together with the unkeeled sphragis of the female, seem to indicate that this taxon was correctly placed by Rothschild (1918) as a subspecies of *japonica* Leech, and it is here so treated.

## ***BHUTANITIS*** Atkinson

*Armandia* Blanchard, 1871 : 809, nota 3. Type-species: *Armandia thaidina* Blanchard, by monotypy. [Homonym of *Armandia* Filippi, 1862.]
*Bhutanitis* Atkinson, 1873 : 570. Type-species: *Bhutanitis lidderdalii* Atkinson, by monotypy.
*Bhutanitis* Atkinson; Bryk, 1934 : 113.
*Bhutanitis* Atkinson; Munroe, 1960 : 13.

### KEY TO THE SPECIES OF *BHUTANITIS* ATKINSON

1 Hindwing upperside with a series of orange marginal internervular markings . 2
– Hindwing upperside without orange marginal internervular markings, being yellow or grey in these areas . . . . . 3
2 (1) Vein $M_3$ of hindwing produced to broad spatulate tail; clasper of male bluntly produced posteriorly and bearing a tuft of thick black pubescence (Text-fig. 14, Pl. 12, figs 92, 93) . . . . . ***thaidina*** (Blanchard) (p. 93)
– Vein $M_3$ of hindwing produced to a narrow tail; clasper of male pointed and bearing sparse pubescence only (Text-fig. 15, Pl. 13, figs 94, 95) ***lidderdalii*** Boisduval (p. 94)
3 (1) Vein $Cu_{1b}$ of hindwing produced to a round lobe; pale bands of wings broad, resembling *Luehdorfia*; female bearing a sphragis (Pl. 12, fig. 91) ***mansfieldi*** (Riley) (p. 94)
– Vein $Cu_{1b}$ of hindwing produced to a distinct tail; pale bands of wings narrow; female without sphragis (Pl. 14, figs 96, 97) . . ***ludlowi*** Gabriel (p. 94)

### ***Bhutanitis thaidina*** (Blanchard)

(Pl. 12, figs 92, 93, Text-figs 14, 25)

*Armandia thaidina* Blanchard, 1871 : 809.
*Bhutanitis thaidina* (Blanchard); Bryk, 1934 : 116.

DISTRIBUTION. China: Shensi, Szechwan, Yunnan.

LARVAL FOOD PLANT. *Aristolochia* sp. (Bryk, 1934).

### ***Bhutanitis lidderdalii*** Atkinson

(Pl. 13, figs 94, 95, Text-fig. 15)

*Bhutanitis lidderdalii* Atkinson, 1873 : 570, pl. 50.
*Bhutanitis lidderdalii* Atkinson; Bryk, 1934 : 118.

DISTRIBUTION. Bhutan. Sikkim. N. India: Assam, Nagaland, Manipur. N. Burma. China: Szechwan, Yunnan.

### ***Bhutanitis ludlowi*** Gabriel

(Pl. 14, figs 96, 97)

*Bhutanitis ludlowi* Gabriel, 1942 : 189.

DISTRIBUTION. Bhutan: Trashiyangsi Valley.

As far as I am aware, the type-series of *ludlowi* Gabriel is unique, no other representatives of this species being known to me.

### ***Bhutanitis mansfieldi*** (Riley)

(Pl. 12, fig. 91)

*Armandia mansfieldi* Riley, 1939*a* : 207, pl. 4.
*Bhutanitis mansfieldi* (Riley); Riley, 1939*b* : 267.

DISTRIBUTION. China: Yunnan.

This species, known to me from the female holotype only, bears a curious resemblance to *Luehdorfia* Crüger in both pattern and wing shape; furthermore it is the only *Bhutanitis* species in which the female bears a sphragis. When more material becomes available, examination of the male genitalia may show whether it has been correctly placed here.

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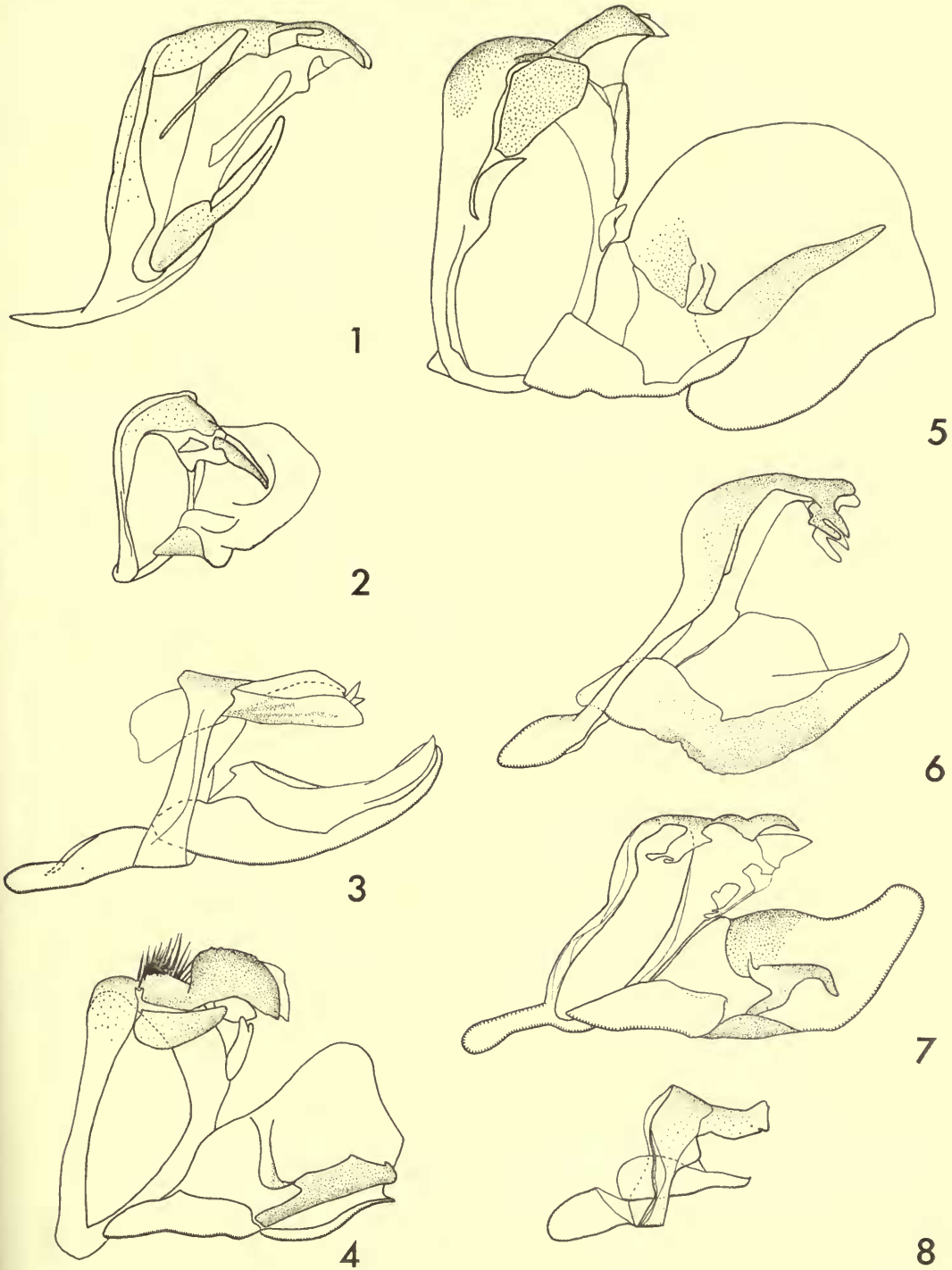
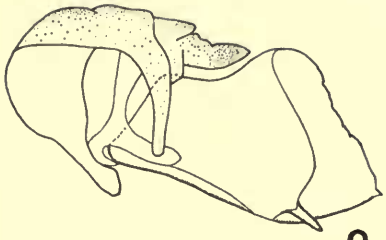



FIGS 1–8. Male genitalia, left clasper removed. 1, *Archon apollinus amasinus* (Staudinger & Rebel). 2, *Hypermnestra helios maxima* Grum-Grshimailo. 3, *Parnassius tenedius tenedius* Eversmann. 4, *P. hardwickii hardwickii* Gray. 5, *P. szechenyii szechenyii* Frivaldsky. 6, *P. glacialis glacialis* Butler. 7, *P. acdestis lathonius* Bryk. 8, *P. simo simonius* Staudinger.



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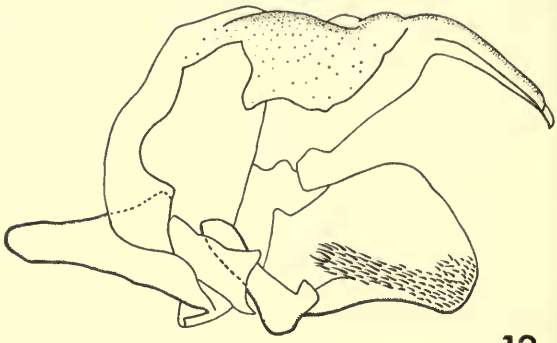

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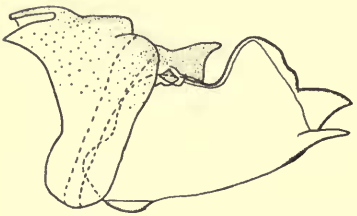

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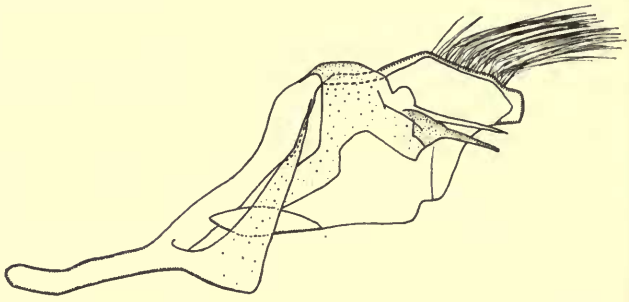

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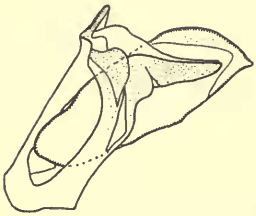

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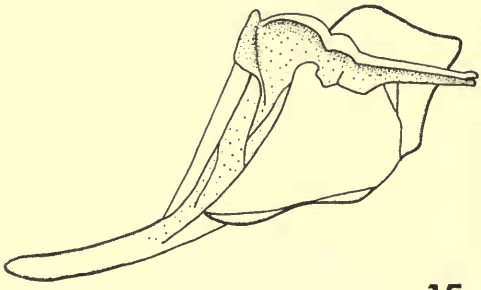

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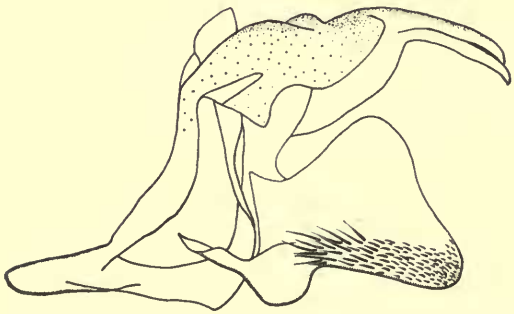

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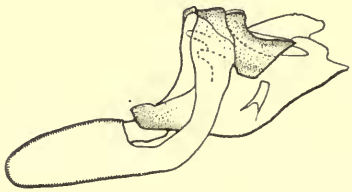

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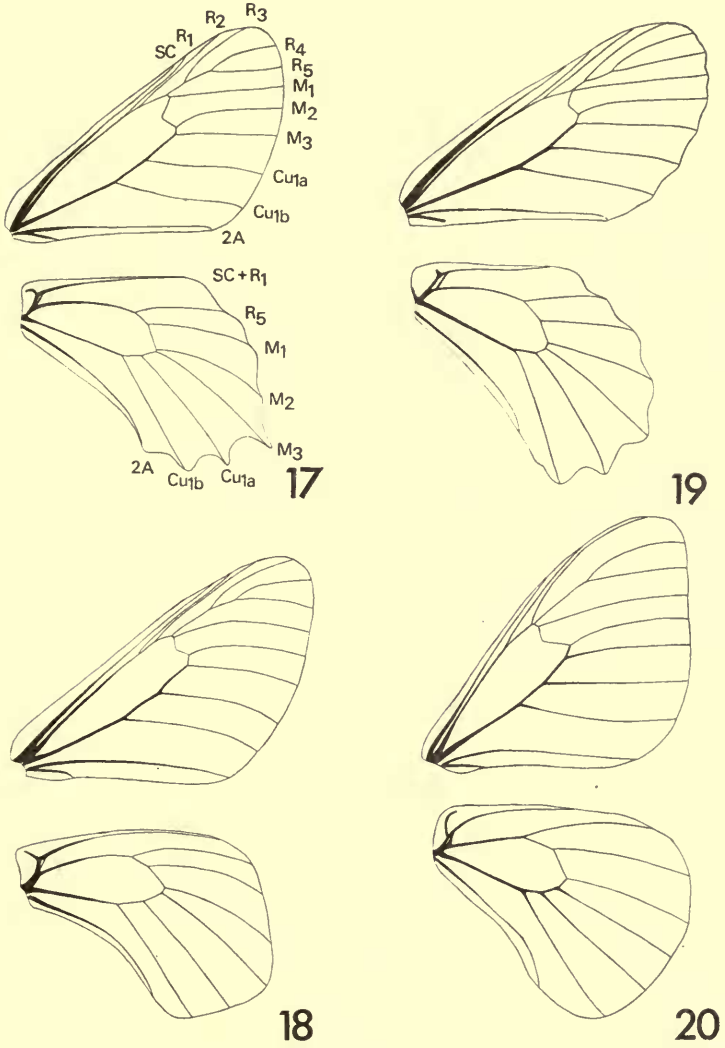
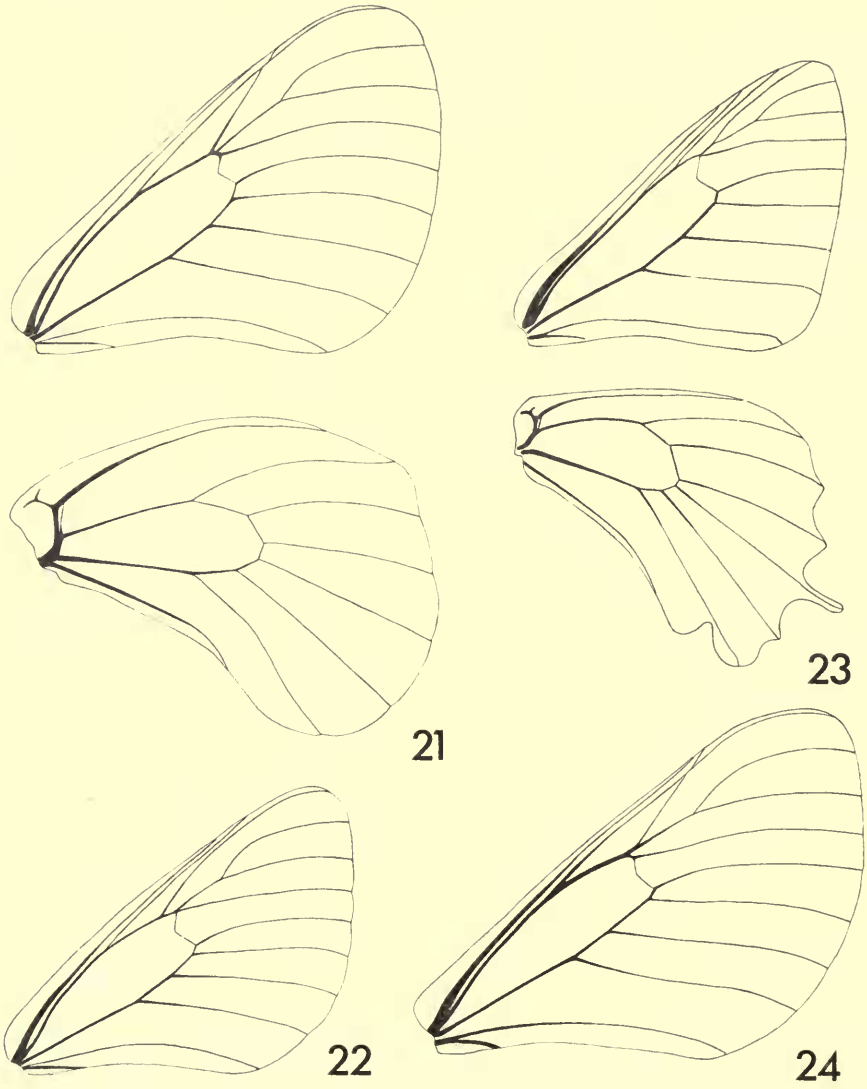
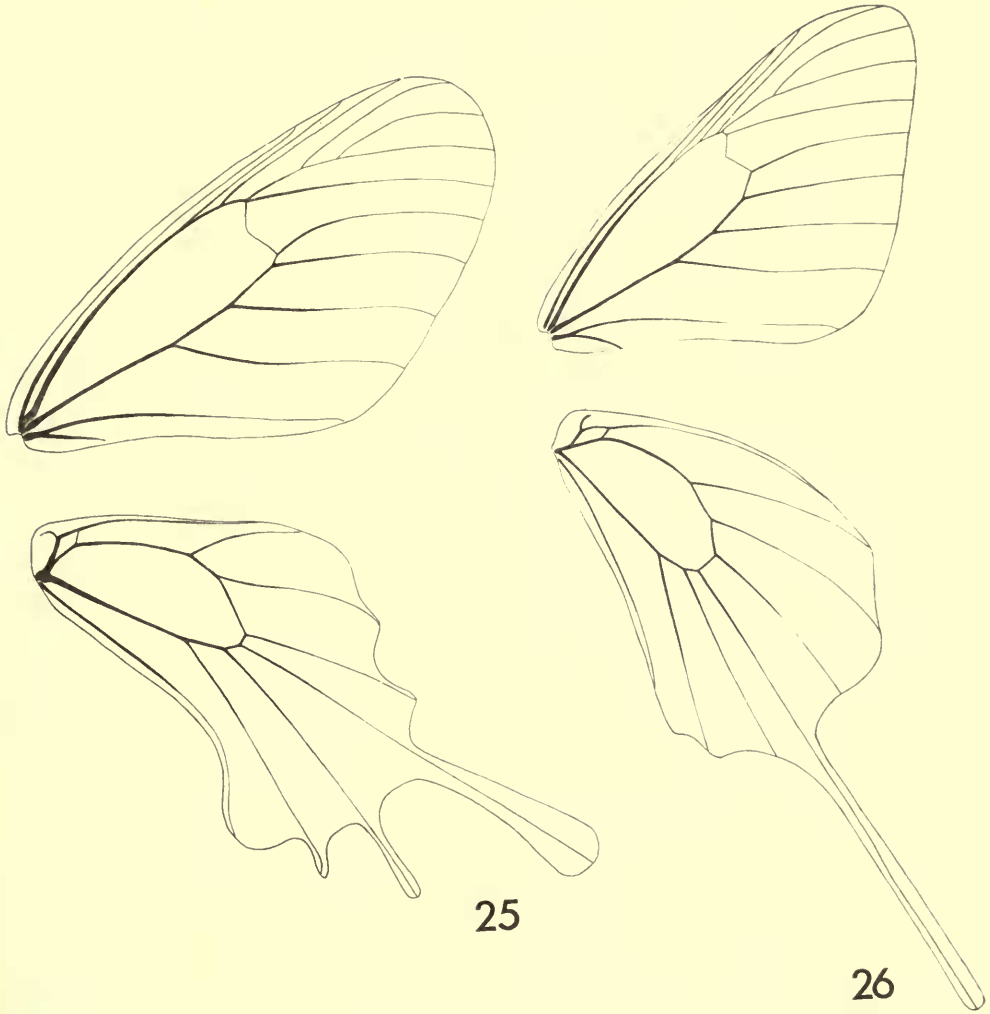
FIGS 9–16. Male genitalia, left clasper removed. 9, *Parnalius polyxena polyxena* (Denis & Schiffermüller). 10, *P. rumina australis* (Esper). 11, *P. cerisy speciosa* (Stichel). 12, *Luehdorfia puziloi puziloi* (Erschoff). 13, *L. japonica japonica* Leech. 14, *Bhutanitis thaidina thaidina* (Blanchard). 15, *B. lidderdalii lidderdalii* Atkinson. 16, *Sericinus montela magnus* Fruhstorfer.



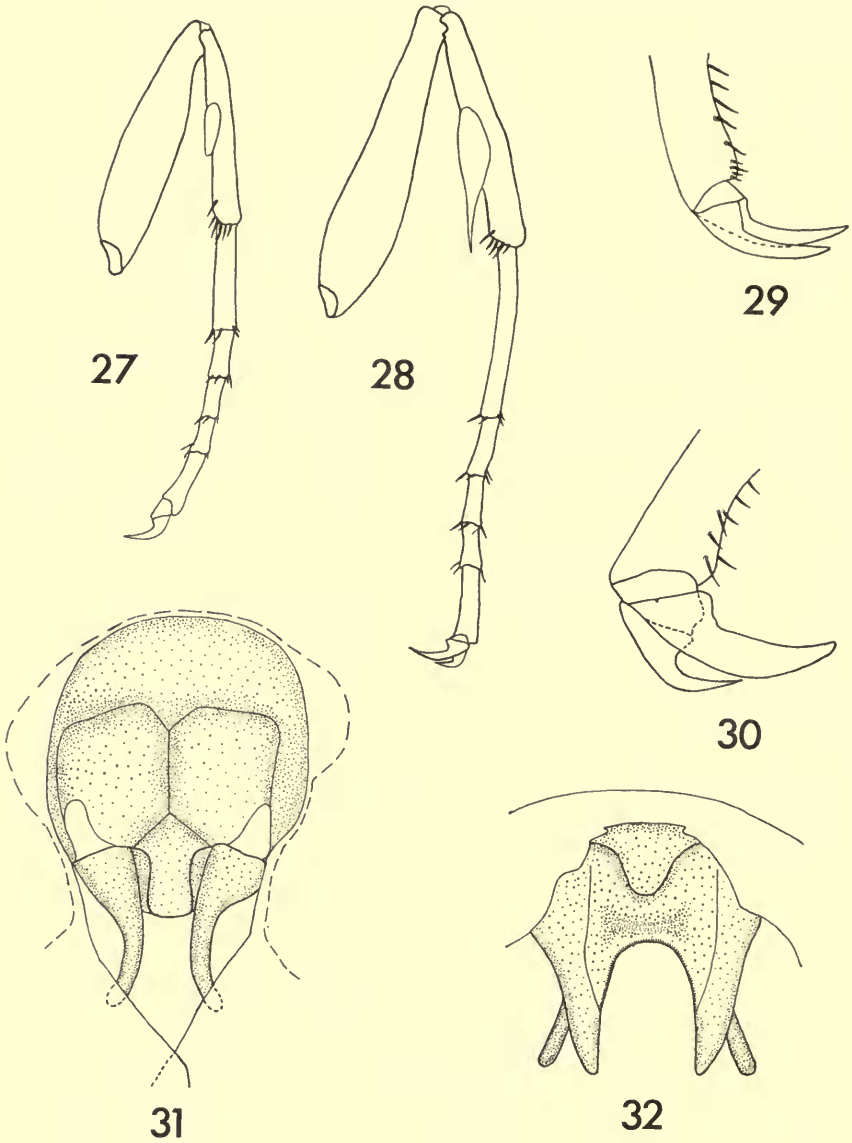

FIGS 17–20. Venation of right fore- and hindwings. 17, *Parnalius cerisy speciosa* (Stichel). 18, *Archon apollinus amasina* (Staudinger & Rebel). 19, *Parnalius polyxena polyxena* (Denis & Schiffermüller). 20, *Hypermnestra helios maxima* Grum-Grshimailo.




FIGS 21–24. Venation of right fore- and hindwings. 21, *Parnassius imperator imperator* Oberthür. 22, *P. cephalus ares* Bryk & Eisner [forewing only]. 23, *Luehdorfia japonica japonica* Leech. 24, *Parnassius charltonius deckerti* Verity [forewing only].




FIGS 25–26. Venation of right fore and hindwings. 25, *Bhutanitis thaidina thaidina* (Blanchard). 26, *Sericinus montela magnus* Fruhstorfer.




FIGS 27–32. 27–28, forelegs, showing foretibial epiphysis of (27) *Parnassius orleans* Oberthür, (28) *P. autocrator* Avinoff. 29–30. Male tarsal claws of forelegs of (29) *Hypermnestra helios* (Nickerl), (30) *Parnassius cephalus* Grum-Grshimailo. 31–32. Dorsal view of uncus of (31) *P. phoebus* (Fabricius), (32) *P. mnemosyne* (Linnaeus).

ring after ring of mud, till the cell is closed in by a lid; the lid is excavated and rendered concave on its outer or upper surface, and is convex and rough on its inner surface, and, in fact, is a simple repetition of the first-formed portion of the cell, a part of a hollow sphere; and if the work proceeded beyond this point, there can be little doubt that this lid of a cell would become the bottom of another similar cell, and thus we should have the cells joined end to end, and with a common straight axis, as we see in the cells of certain other bees—*Megachile* for instance; here, however, the cells are deposited in cylindrical burrows made in the ground, and the cells themselves are cylindrical. I have witnessed the construction of the first part of the cell of the *Osmia*, and I have seen cells in all states of progress from that part to their completion. The completed *Osmia* cell strongly reminded me of the isolated cells built by the hive-bee for the queen-bee larva; the general form is the same to this extent,—they are both hemispherical at the base, and gradually approach the cylindrical form towards the mouth of the cell.

I have still one other form of cell, to which I wish to direct your attention. These two cells, like those of the *Osmia*, are constructed of mud, but the insect that formed them (a species of *Eumenes*) belongs to the wasp tribe. Whilst the *Osmia* is a short stout insect with short legs, the *Eumenes* is slender and has long legs. The cell of the *Osmia* would be completely filled by the pupa of the insect reared in it; but that of the *Eumenes* is much larger when compared with the size of the insect that constructed it. This difference in the proportionate size of the cells has, no doubt, reference to the kind of food which has to be stored up for feeding the larva. Furthermore, these cells differ from those of the *Osmia* in being almost perfectly spherical. They remind one of certain water bottles, and, like them, have a short neck, through which is the opening into the nest. The opening is very small, but I have little doubt would permit the insect to enter the cell; but, on the other hand, I much doubt whether she could perform the whole of the work of its construction from the outer side. I believe, indeed, that in executing the latter part of the work, at least, the insect was inside the cell. However this may be, here is a cell, the foundation of which was laid down in segments of circles of much larger size than those seen in the cell of the *Osmia*, and, in connexion with this, we have the fact, that the comparatively long legs of the *Eumenes* would give it the power of a far greater stretch, supposing that her work was com-

It is extremely difficult to observe the cilia which lie at the bottoms of the furrows (*mf*). Wallengren (*l.c.*) ascribes to them the duty of carrying lengthwise of the furrow to the lower margin of the palps the minute particles that may fall between the ridges. But Siebert ('13) thinks they lead in the opposite direction.

In the event that any particles get past the palps they may still be rejected at the mouth. A strong compression of the lips will force them outward to the edge of the lips, where they encounter the cilia directed backward (Figs. 3 and 4, lower margin) and are carried to the edge of the palps and dropped into the mantle chamber.

The outer surfaces of the palps and lips have as their function the removal of particles from the mantle chamber (Fig. 4).

McAlpine's (*l.c.*) observations upon the movements of detached parts led him to conclude that the palps and gills have nothing to do with feeding, and that they are concerned only with carrying away foreign material. But Wallengren's (*l.c.*) conclusions are based upon far more careful and logical experiments, and Siebert's (*l.c.*) recent paper on the epithelium of Anodonta is of a confirmatory nature.

## Observations.

The ciliary currents may be observed quite readily on a mussel from which the shell and mantle of one side have been removed, or on detached parts, which, as stated, continue to exhibit ciliary activity for a long time. Small quantities of carmine, indigo, or other nearly neutral coloring matter may be dropped upon the part to be studied, and their behavior noted. Care must be exercised in the amount of water used. The less water the better, within limits, for in a large amount of water currents may obscure the action of the cilia. The surface of the organ must be level in order to offset gravitational disturbance. A small piece of any ciliated organ, when placed in a watch glass with water and a very little color, will show under low power both the cilia and their currents in great detail.

### *The Ciliary Streams.*

The figures will show more clearly than description the course of the streams of material collected from the water. All the

ciliary currents of the inner gills (Figs. 1, 2, 4, 6, and 7) are seen to be directed downward. When they reach the lower edge of the gill they pass around the points of the lamellæ to the under side. Here the lamellæ of the two faces of the gill form an inverted trough (Fig. 7, *tr*) in which the particles small enough to be used as food may be carried to a point just above the labial palps (Figs. 1 and 2, *X*). At this point they accumulate in strings of mucus until their weight causes them to fall into the mantle chamber, or into the trough formed by the upper margins of the palps. This depends upon the desirability of the material for food, or rather upon the presence or absence of unfavorable stimuli, and is probably regulated by reflexes, since the palps are quite motile, even after being severed.

The ciliation of the outer gills tends upward (Figs. 1 and 6). When particles carried upward by their inner surfaces reach the top, they are passed over to the inner gill, and thence to the point described above. But the gleanings obtained by the outer surface of the outer gill pass over to the mantle at the line of their attachment (Figs. 1 and 6). All material transmitted by the outer gill and collected by the uppermost part of the mantle is removed forward by the latter to a point just above the attachment of the palps to the mantle and body wall. So long as the upper edge of the palps remains applied to the mantle this material passes into the trough between the palps, backward around the line of attachment of the two palps, then forward again between them (Figs. 3 and 4). In case of an unfavorable stimulus the palps are withdrawn from the mantle at this point the material glides past them and is carried downward and backward by the cilia of the lower part of the mantle to the region of the excurrent siphon (Fig. 1). All the cilia of the lower part of the mantle are directed toward the posterior. So are those of the epithelium of the lower body wall. Their function is that of collecting particles to be thrown out. A small portion of the body wall, near the upper margin, is ciliated like the same portion of the mantle, and must be concerned with the collection of food.

From the foregoing account and from the figures it is evident that the region of the labial palps is the center toward which all the ciliation of the upper part of the mantle chamber tends. All

material gathered by both gills and by the dorsal part of the body epithelium and mantle must finally reach one of the three adjacent points—(1) the mantle just above the palps, (2) the body wall just opposite the first point, or (3) a point on the edge of the inner gill just above the labial palps. All these are within easy reach of the palps (Figs. 1, 2, 4, and 6).

No one, to my knowledge, has succeeded in inducing a mussel to behave normally, after the shock of removing parts of the shell and mantle in order to observe the palps at work. But I have repeatedly obtained the reactions which occur. When the palps lie in contact with either body, mantle, or gill, their collections of material pass between the palps and mouthward. Otherwise such material is carried on down by the several structures and discarded. The fact that the upper margins of the labial palps adhere to each other and form a trough (Figs. 4 and 6) makes it possible to reach at least two of the three sources of supply simultaneously.

Since we have the mechanism for such a method, and since the reactions, though fragmentary and under abnormal conditions, are of a confirmatory nature, we may safely infer that the labial palps do actually accept or refuse food, either through reflex stimuli or in response to volition.

### *The Function of the Mucus.*

The entire epithelium touching the branchial chamber is abundantly supplied with glands which secrete a mucous substance (Siebert, *l.c.*). The mucus envelops and binds together in strands the material to be transported by the cilia. This is particularly true of those particles which are of a very distasteful nature. That this secretion is dependent on local reflexes is quite evident from the fact that it may be stimulated in an organ entirely severed.

It is this collection of food in a film of mucus, which makes possible the mechanism of the furrowed surface of the labial palps. If each particle were manipulated independently, it would tend to eddy back and forward between the opposing streams of cilia, and considerable confusion might result. But a strand of material spans the summits of several ridges, and

while touching cilia that lead in both directions, it obeys the ciliary streams which lie uppermost and exert the greater force upon it.

*Conclusions on the Function of the Ciliary Currents.*

The surfaces of the gills and of the upper part of the mantle, and the contiguous faces of the labial palps, in fact, nearly all the upper parts of the mantle chamber, have for their general purpose the carrying of food to the mouth. The lower part of the mantle chamber, upon which the heavier fragments are likely to fall, are concerned principally with removing undesirable matter from the animal.

THE SELECTION AND REJECTION OF FOOD PARTICLES.

Observers have differed widely in their notions of the ability of the mussel to select its food. To me it is evident that there are, to summarize, four points where such choice is exercised:

(1) The labial palps, at the upper margin.
(2) The labial palps, on the furrowed surfaces.
(3) The mouth.
(4) The incurrent siphon.

As to the last, it is surrounded by a row of pointed, fleshy papillæ, having a resemblance to plant structures. These have two sensory functions—tactile and gustatory; for upon being disturbed mechanically they are withdrawn into the shell, while a continued teasing, or a strong chemical stimulus results in the closing of the shell, or perhaps only the siphons.

It is true that some material of no food value finds its way into the alimentary canal. But the quantity is far smaller than if no selection were made, and is of a harmless nature. All distinctly injurious substances are rigidly excluded.

That which has been rejected at the mouth, palps, or gill accumulates upon the lower posterior margin of the mantle or body wall, along with the collections made by these parts themselves, and is here massed in clots of mucus. When this has attained considerable size the animal ejects it with a rapid current of water, set in motion by a quick contraction of the adductor muscles and closing of the shell (Figs. 1 and 2, *r*).

## The Rate of Siphoning.

An effort was made to determine the rate at which water is siphoned through the mussel. If this can be done it will contribute to several quantitative studies relative to the feeding habits, and that of the effect of temperature and other conditions upon the activity of the cilia.

But it is very difficult to attach apparatus for making measurements to the siphons of the animal. Intimate contact must be made to avoid leakage and a high per cent. of error. Such contact irritates the mussel, so that it does not behave normally.

In only one mussel did I succeed in obtaining what seemed a normal circulation of water, when under this annoyance. This was done by placing a short piece of soft rubber tubing in the excurrent siphon. Into the end of this was thrust a calibrated glass tube, having a capacity of 2 c.c. between two given marks. The point of a pipette containing neutral coloring matter was thrust into the rubber just outside the siphon. The mussel with this simple apparatus was put into an aquarium near the lake, where the water could be changed frequently and the lake conditions maintained. A touch upon the pipette released a drop of coloring matter into the tube, where it encountered the stream flowing from the excurrent siphon.

This individual was a *L. luteolus* weighing 200 grams with the mantle chamber filled. It required five seconds for the pigment to pass between the two marks upon the tube, whenever the incurrent siphon was opened fully. The reading was repeated a number of times at intervals, with the same result.

While these are but meager results, they give at least an idea of the volume of water siphoned. At the above rate there are siphoned 24 c.c. per minute, 1,440 c.c. per hour, or 34,560 c.c. per day. To filter a liter of water would require 42 minutes.

## Digestion.

Digestion fluctuates more in the case of mussels than does their feeding. That is, the animal continues to feed regardless of appetite; but the degree with which the food so ingested is really made use of seems to depend upon the relation of supply and demand. At times nearly all the intestinal contents are found

to be at least partially digested; while again much material is found, even in the rectum, in perfect preservation, and often the fæces themselves contain forms which are apparently unaffected. Hence we may conclude that appetite fulfills its function by the control of the secretion of the digestive juices, without the voluntary regulation of the food supply.

## The Crystalline Style.

The literature on the lamellibranchs is particularly rich in speculations concerning the function of the crystalline style; but I cannot forbear adding a word here parenthetically on the subject, by calling attention to the excellent work done by Mitra ('01) upon it. He reviews all the previous theories and repeats the experiments, extends them, and brings physiology, chemistry, and comparative morphology to bear upon the matter, so that there seems to be no way of escaping his conclusion that the style is a digestive ferment which converts starch into sugar. The previous hypothesis of Gegenbaur, that it is a secretion of enteric epithelium, he holds to be true, but says that this does not account for its existence. Against Balfour's notion that the style is a rudiment of a radular sac he brings six weighty proofs, and dismisses with two objections each the theories of Claus and Sedgwick, that it is an excretion product and a reserve of nutriment, respectively.

In two minor particulars my observations do not agree with those of Mitra. (1) In an experiment upon the renewal of the style he concludes that it appears and disappears *periodically*. But from the description of his experiment we find that the water and food supply were renewed at regular intervals, whence the periodicity. In a similar experiment of my own the crystalline style was found to disappear only with the lack of food, and to be regenerated only when food was supplied, regardless of time. Then too, as long as the food supply is abundant the style is never wanting. In all mussels freshly removed from the lake it was found to exist. In all mussel freshly removed from the lake it was found to exist. In these matters the work of Haseloff ('88) also confirms my point.

Hence the food supply must be a factor in its secretion. As it

is dissolved when food is lacking, and as it is a proteid, why is not Sedgwick's theory as to its being a reserve food supply also true?

(2) The presence in the core of the crystalline style of cells similar to those found in the liver epithelium leads Mitra to conclude that the liver is the probable origin of the structure. This also does not seem well substantiated, especially in species where the style is found in a diverticulum. No channel for the passage of a secretion from the liver to the seat of the style has been discovered, and the ciliation of the stomach and intestine forbids their aiding in its transmission.

Since the publication of the above paper Grave ('03) has suggested in his work on the oyster that the crystalline style may perform the duty of preventing coarse particles from passing through the digestive canal. In fresh water mussels I can see but one way in which the style may attend to that function—by digesting the said particles. There are at least two objections to this explanation for fresh water mussels (1) As we have seen, the animal is well protected against the entrance of such particles. (2) In case they were admitted to the stomach but kept out of the intestine, they would accumulate in the stomach, for it is not equipped with either a muscular or a ciliary system by which these could be expelled through the mouth. Then too, the mouth is no larger than the intestine and no more capable of receiving them. The largest body I have seen in the alimentary tract was a fragment of *Oscillatoria* (or similar form) measuring 1.5 mm. in length.

## Food Materials.

No one but Zacharias ('07) seems to have undertaken a detailed examination of the contents of the alimentary tract of the freshwater forms, so I give the results of a purely qualitative examination. In marine mussels it is said that the food consists almost altogether of minute plant forms, and of these almost all are Diatomaceæ. In the mussels which I examined I found a somewhat different condition. In the first place there is a little higher proportion of animal food present. Living animals are found but rarely, and most of these are apparently living transiently upon the contents of the tract. But the mussel does

not refuse minute dead animals or small fragments of sloughed and decaying animal tissue. In many cases bits of material seemingly the carapaces of small crustacea are found to resist digestion throughout the alimentary canal. However most of the animal matter consists of shapeless unidentifiable fragments.

In order to determine the ability of mussels to capture and digest living animals, a few were kept without food for several days, then a rich culture of *Paramecium* was added. This was found to be of sufficient nutrient value to regenerate the crystalline style in part. The digestive tract was seen to be filled again with half assimilated material. Few living *Paramecia* were discovered beyond the stomach, thus corroborating Vogt and Jung's statement ('94) that digestion is nearly completed in that organ.

In the second place, the plant material does not consist so largely of diatoms, there being probably as many other algal forms. Very few desmids were observed, in fact only three genera which I could identify with certainty. But Winona Lake does not produce many desmids, and it is not probable that they are discriminated against where they exist more abundantly.

The following is a list of genera recognized. There were several more which I could not identify.

| Diatomaceæ. | Other Algæ. |
|---|---|
| *Amphora* | *Anabæna* |
| *Arachnoidiscus* | *Aphanocapsa* |
| *Cocconeis* | *Cœlastrum*[1] |
| *Cocconema* | *Cylindrocapsa* |
| *Coscinodiscus* | *Eudorina*[1] |
| *Craspedodiscus* | *Glœocystis* |
| *Cymbella* | *Leptothrix* |
| *Epithemia* | *Lyngbya* |
| *Fragilaria* | *Merismopedia*[1] |
| *Gomphonema* | *Œdogonium*[1] |
| *Melosira* | *Oscillatoria* |
| *Navicula* | *Pandorina*[1] |
| *Pleurosigma* | *Pediastrum*[1] |
| *Surirella* | *Protococcus* |

*Synedra*
*Triceratium*
DESMIDACEÆ.
*Closterium*
*Netrium*
*Staurastrum*[1]
*Rhaphidium*
*Scenedesmus*
*Spirogyra*
*Tetraspora*
*Ulothrix*
*Vaucheria*

MISCELLANEOUS CONTENTS.

Inorganic fragments,
Plant and animal debris,
Mold,
Ova and spermatozoa
  Of other animals,
  Of the same individual or species,
    (The sperm living and in motion),
Spores and swarm spores.

The posterior half of the mussel shell, the part protruding above the substratum, is usually very richly encrusted with diatoms and other algae. This may serve as a private garden, and particles dislodged by the passage of the animal along the bottom thus be brought into the incurrent siphon.

In addition to the very valuable assistance which I have received from Prof. Scott, I wish to acknowledge by indebtedness to the John Crerar Library of Chicago, through which I was able to procure several useful references, and to Profs. C. H. Eigenmann and W. C. Curtis, who have made indispensible critical suggestions.

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[1] Also noted by Zacharias (*l. c.*).

## XXII. *Descriptions of some Coprophagous Lamellicorn Beetles from New Holland.* *By* J. O. Westwood, F.L.S.

[Read 1st November, 1841.]

The insects described below belong to that division of the family of the sacred beetles, (*Scarabæidæ*, MacLeay, *Ateuchites*, auct. al.,) which is distinguished by having the head and thorax destitute of horns; the scutellum obsolete; the intermediate tibiæ with two spurs, and they, as well as the posterior tibiæ, not dilated into an elongated triangle at the tip, as in the *Coprides*.

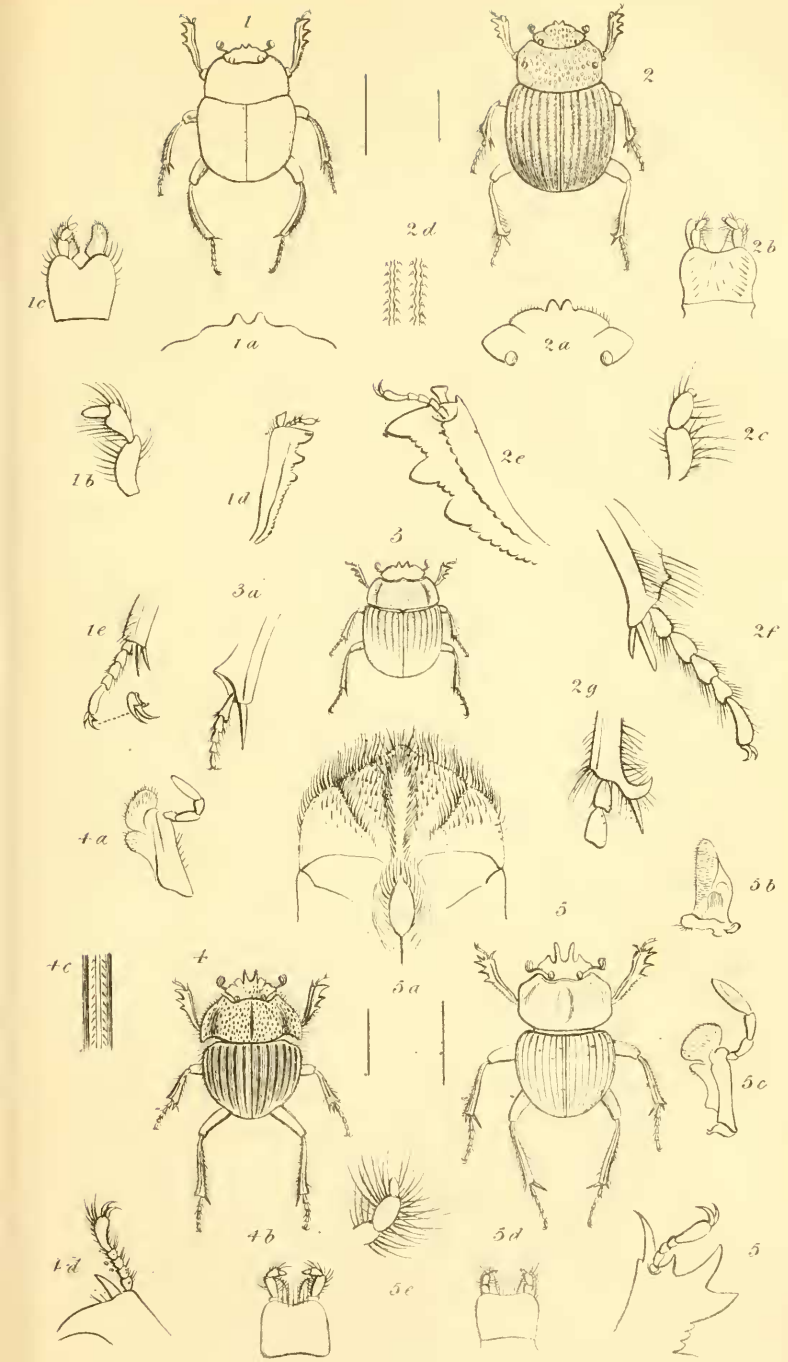
It is a remarkable peculiarity in Entomo-geography, that whilst the arid deserts of Africa produce great numbers of coprophagous *Scarabæidæ* of large size, New Holland is almost entirely destitute of these insects; and of these, the largest appears to be the

*Circellium hæmisphericum*, Péron, (Pl. VIII. fig. 3,)

figured by Guérin in the "Iconographie du Règne Animal, Insectes," pl. 21, fig. 3, (and described in the texte, p. 76,) as $7\frac{1}{2}$ lines (15 millem.) long. This insect has been formed by Reiche into the genus Coproecus, in the "Revue Zoologique, par la Société Cuvierriene" for July in the present year (1841, p. 211).

M. Reiche, in his memoir on this group of insects, published since the above was written, states that in the specimen belonging to the Jardin des Plantes the antennæ and part of the trophi are wanting. His figure of the insect is much more elongate-oval than that of M. Guérin; it is, however, evidently not broad enough, according to the dimensions given by M. Reiche. This author has detected short but distinct tarsi in the fore feet, and a single spur at the extremity of the middle tibiæ. He has, however, represented the posterior tibiæ as terminating on the inside in an acute spine as long as the calcar; whereas M. Guérin figures it as truncated, obliquely emitting the calcar near the middle of the truncation: and he describes the elytra as very convex, with six elevated smooth costæ, each of which is accompanied on each side by a row of small punctures, in which respect it approaches *Tessarodon*, from which, however, it is separated by its possessing only one spur to the middle tibiæ.

Another species, remarkably distinct in having the hind angles of the thorax acute and prolonged backwards, and being $3\frac{1}{4}$ lines long, has been mistaken for the *Ateuchus Hollandiæ* of Fabricius, by Dejean, (who has formed it into the genus Aulacium, adopted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by Reiche in the work above referred to,) whilst De Laporte formed it (with the same erroneous specific name) into his slightly characterized genus *Mentophilus* (Hist. Nat. An. art. Col. vol. ii. p. 74, pl. 4, fig. 4). Reiche has, however, proposed for it the specific name of *A. carinatum*. (Pl. VIII. fig. 4, and details.)

The real *Ateuchus Hollandiæ*, the original specimen of which, described by Fabricius from the Banksian Cabinet, I detected amongst the unarranged insects of that collection in the possession of the Linnæan Society, although still authenticated by the original label, has been formed by Mr. Hope into the genus *Tessarodon*, in his "Coleopterist's Manual," vol. i. p. 55; the insect itself being figured in his plate 3, fig. 15. More recently Mr. Hope has obtained two other species of the same genus from Australia, which have enabled me to give the following generic details; but as these insects absolutely disagree with the character expressed by the name *Tessarodon*, (having only two teeth in the front of the clypeus,) another name ought to be given to the genus.

TESSARODON, Hope, Reiche.

*Corpus* obovatum, subconvexum. *Caput* anticè in dentibus duobus triangularibus brevibus productum; angulis posticis posticè haud porrectis. *Mentum* subquadratum, anticè parum emarginatum. *Palpi* labiales breves, articulis duobus basalibus subæqualibus longe setosis, tertio (apicali) minimo. *Prothorax* rudè punctatus, lateribus in medio angulatis vel subangulatis, utrinque versus marginem profundè impressus. *Elytra* ovato-rotundata, tenuissimè striata, striis per paria ordinatis, singulo pari utrinque lineâ punctorum impressorum notato. *Pedes* longi. *Tibiæ* anticæ calcari brevi obliquè truncato instructæ; *tibiæ* intermediæ curvatæ depressæ, pone medium subdilatatæ, angulo apicali externo obliquè truncato, bicalcaratæ; *tibiæ* posticæ minus curvatæ, apice interno recurvo unicalcaratæ. *Tarsi* antici brevissimi, articulo ultimo longo; *tarsi* 4 postici longiores, articulo 2ndo præcedenti parum majori; unguibus parvis, valde curvatis.

Sp. 1. *Tessarodon Hollandiæ.*

T. subrotundatus, totus ater, clypeo quadridentato, prothorace punctis oblongo-ovalibus impresso, elytris sulcatis, striis per paria impressis.

Long. corp. lin. $2\frac{2}{3}$.

Habitat in Nova Hollandia.

In Mus. Soc. Linn. Lond., olim Banks.

Syn. *Scarabæus Hollandiæ*, Oliv. Ins. l. 3, 174, t. 13, fig. 119; Fabricius, Ent. Syst. 1, p. 65.
*Ateuchus Hollandiæ*, Fabricius, Syst. El. 1, p. 57.
*Tessarodon Novæ Hollandiæ*, Hope, Col. Man. 1, p. 55, pl. 3, fig. 15; nec *Aulacium Hollandiæ*, Dejean, vel *Mentophilus Hollandiæ*, Lap.

This species is smaller and (in proportion to its size) broader than either of the two following; the sides of the head are produced on each side of the two middle horns into an advanced conical lobe; the club of the antennæ is pale yellow; the fore tibiæ are not so broad as in the next species, and the teeth on the outside are obtuse; the hind tibiæ appendiculated at the tip on the inside.

The above description, and the figure published in Mr. Hope's Coleopterist's Manual, were taken from the original specimen still preserved at the Linnæan Society.

Sp. 2. *Tessarodon angulatus*, Westw. (Pl. VIII. fig. 2.)

T. subovalis, obscurè castaneus, capite et prothorace rudè punctatis, clypeo in medio dentibus duobus conicis, lateribus ante oculos valde angulatis, tibiis posticis ad apicem appendiculatis.

Long. corp. lin. 3.

Habitat in Nova Hollandia apud Swan River?

In Mus. D. Hope.

This species is longer but not so broad as the typical species: it is entirely of a dark castaneous colour; the head with two conical flat spines in the middle, on each side of which the margin is rounded for a short distance, it then runs nearly straight to a very sharp angle in front of the eyes; the club of the antennæ is pale fulvous; the head and thorax are very strongly and irregularly punctate, and the elytra are rather deeply striated in double rows, with punctures on each side; the anterior tibiæ are furnished with a short spur dilated and truncated at the tip; the hind tibiæ are appendiculated on the inside at the tip.

Sp. 3. *Tessarodon piceus*, Hope.

T. parvus, subovalis, castaneus; capite et prothorace rudè punctatis, capitis angulis ante oculos rotundatis, tibiis posticis simplicibus.

Long. corp. lin. $2\frac{1}{2}$.

Habitat Port Essington, New Holland.

In Mus. D. Hope.

Syn. *Tessarodon piceum*, Hope, MSS.

## EXPLANATION OF PLATE II

FIG. 3. The inner labial palp; outer palp removed; normal ciliary streams of furrowed surface.

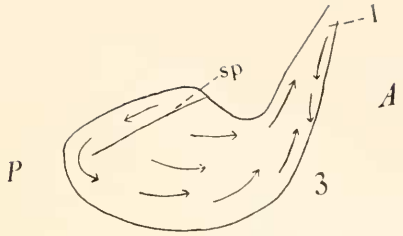
FIG. 4*a*. (Slightly diagrammatic. See Fig. 4*b*.) The left labial palps, adjacent mantle surface, and end of gills; right mantle, gills and palps, and whole body removed; showing point (*X*) where streams of food from gills and mantle converge at the labial palps.

Broken arrows indicate the ciliary currents of the mantle; solid arrows those of the gills; and barbed arrows those of the palps. Where the broken arrows are seen upon the gills they are meant to apply to the mantle just beneath.

FIG. 4*b*. Key to Fig. 4*a*; all organs removed except left mantle, gills and palps; the area within double lines has been enlarged as Fig. 4*a*.

*Abbreviations.*

*A*, anterior,
*aa*, anterior adductor muscle,
*fs*, furrowed surface of labial palps,
*gi*, inner gill,
*go*, outer gill,
*l*, lips
*lp*, labial palps,
*lv*, liver,
*ma*, unfurrowed margin of palps,
*ms*, left mantle,
*P*, posterior,
*pa*, posterior adductor muscle,
*r*, mucous accumulations of refuse material,
*sc*, suprabranchial chamber,
*se*, excurrent siphon,
*si*, incurrent siphon,
*sp*, line of attachment of the palps to each other,
*X*, point of convergence of ciliary currents near labial palps.

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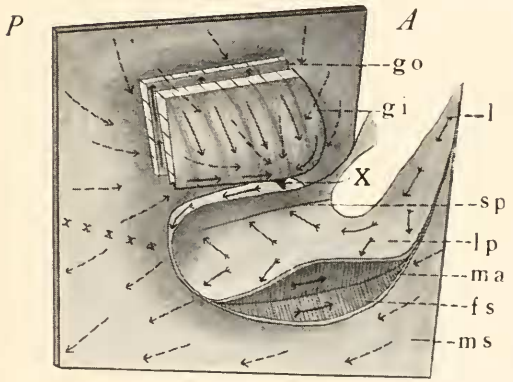


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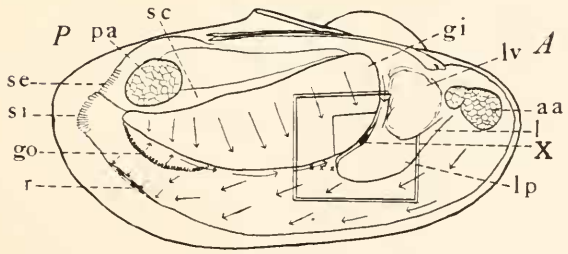



4b

## EXPLANATION OF PLATE III.

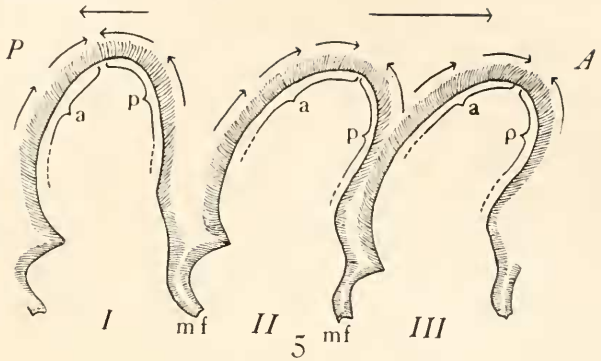
FIG. 5. Cross section of ridges on contiguous surfaces of labial palps (Fig. 4*a*, *fs*) showing their several positions. While *a* is uppermost (as in *II* and *III*) material is carried mouthward; when *p* is raised by the erection of the ridge (as in *I*) the stream is reversed toward the posterior, and the cilia *a* no longer function.

FIG. 6. (Slightly diagrammatic.) Cross section of the ciliated organs concerned with food collecting; showing how the labial palps (*lp*) by occupying several positions in the mantle chamber, may or may not receive the material collected at *X*. As a matter of fact, the palps can span almost the entire width of the mantle chamber at this point, but the width is exaggerated here to show details with greater clearness.

FIG. 7. (After Posner.) Cross section of the edge of inner gill; showing the groove (*tr*) by which material is transported toward the palps.

*Abbreviations.*

*I*, ridge erected; current reversed,
*II–III*, normal position of ridges,
*A*, anterior,
*a*, cilia directed anteriorly,
*f*, foot,
*gi*, inner gill,
*go*, outer gill,
*ic*, interlamellar chamber,
*lp*, labial palps,
*m*, mantle.
*mf*, cilia at bottom of furrows,
*P*, posterior,
*p*, cilia directed posteriorly,
*r*, mucous accumulations of refuse material,
*tr*, trough at margin of inner gill,
*X*, points of convergence of ciliary currents near labial palps.

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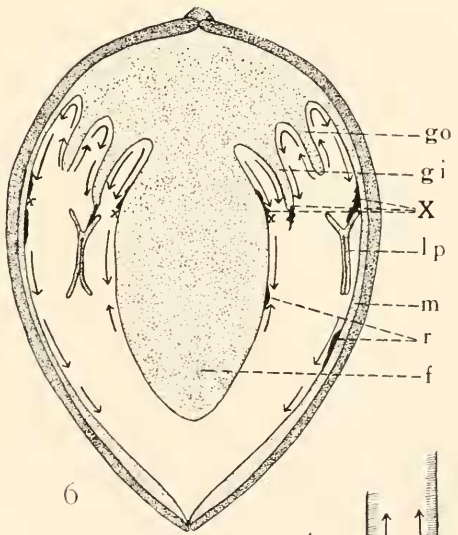


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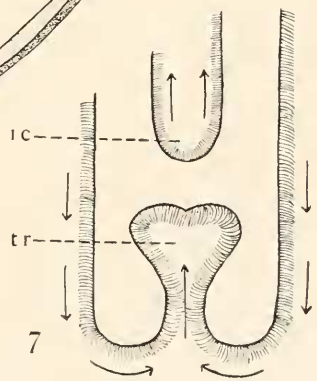


7

# THE MATURATION DIVISIONS IN ASCARIS INCURVA.

H. B. GOODRICH.

In the following preliminary account of the spermatogenesis and ovogenesis of a nematode, *Ascaris incurva* Rud., it is intended to call particular attention to a remarkable XY-group that forms the extreme case thus far observed. The X-group consists of no less than eight components of which one is a vestigial microsome, while the Y is represented by but a single chromosome. The nearest approach to this case is that described by Edwards, '10, for *Ascaris lumbricoides*, where in the heteropolar mitosis the X-element is found to consist of five components unmated by a Y. The extreme example hitherto described in which a Y exists is that of *Acholla multispinosa* described by Payne, '10, in which case five X-components are opposed by one Y, but here the Y is equal to or larger in mass than the combined X-elements.

*Ascaris incurva* is a parasite found in the stomach of the sword-fish, *Xiphias gladius* L. The material was collected at Woods Hole during the summer of 1913, and most of this thus far studied was fixed in strong Flemming's or Gilson-Carnoy's fluid.

A study of the spermatogonial cells has proved unsatisfactory as the chromosomes are closely massed and the cytoplasm stains deeply. Counts vary from 33 to 35. Fig. 1, showing a cell somewhat over-extracted as is desirable to give the necessary contrast, gives a count of 35 chromosomes including the microsome.

During the growth stages a part of the chromatin is massed in a large irregular karyosome. Late prophase or metaphase figures of the first spermatocyte division show 21 chromosomes or 22 if the Y is widely separated from its mate in the X-group (Fig. 2).

Early anaphase figures of the first spermatocyte division show most clearly the unequal nature of the separation of the chromosome groups. Thirteen autosomes lying at or near the periphery

of the plate divide equally, thus forming two anaphase plates of thirteen chromosomes, typically arranged in a ring except that at a point of one daughter plate a gap is observed, opposite which in the other plate is a fourteenth chromosome (Figs. 4 and 6). There remain eight chromosomes lagging in the center of the spindle and arranged in a characteristic plate consisting of six chromosomes of the average size, the microsome and a larger long chromosome arranged in an approximately oval or circular plate with the long chromosome projecting from the periphery (Figs. 5 and 8). As the daughter plates separate, this peculiar group tips, apparently as a unit, so that the long chromosome approaches the gap in the ring of thirteen autosomes. Eventually this whole group passes to the center of the ring and thus the two daughter-cells (second spermatocytes) receive respectively 14 and 21 chromosomes. Size relations and position facilitate the identification of homologous daughter chromosomes of the anaphase plates when these are observed superimposed within a single section (Figs. 4 and 6). Thus the thirteen autosomes of either daughter ring may be readily identified, and, by elimination, the fourteenth of one ring unmated in the other. This fourteenth chromosome must therefore be considered as a Y-chromosome mated by that member of the X-group, the long chromosome, which is first inserted into the gap of the one ring, corresponding to the space occupied by the fourteenth chromosome of the other. Side views of metaphase figures of this division (Fig. 3) show the Y-chromosome lying opposite one end of its longer mate to which it may be united. Fig. 3 is an optical section of such a group showing the Y, its long mate, six other elements of the X-group massed and undivided, surrounded by certain of the dividing autosomes. A cleft in the X-chromosomes indicating the line of division in the ensuing second spermatocyte division may often be observed in the anaphase stage of the first division (Fig. 8). The long chromosome splits lengthwise and usually the chromatin appears concentrated at either end of each half giving a quadrivalent appearance and suggesting a tendency to separate in two parts, one to remain the mate of the Y and the other to increase the number of those unmated.

From the foregoing it will be clear that the secondary spermato-

cyte divisions should be of two classes, one showing 21 chromosomes including the microsome, and the other 14. Examination of numerous metaphase plates has proved this in the clearest manner to be the case (Figs. 9 and 10). This condition may be compared with that in *Ascaris lumbricoides* in which the two classes of secondary spermatocyte cells show respectively 19 and 24 chromosomes, or with *Acholla multispinosa* in which the spermatids receive either 11 or 15 chromosomes.

Oogonial cells showing division figures have not been found but the constant presence of 21 chromosomes in the maturation divisions of the egg indicates most certainly that the diploid number in the female is 42. Metaphase and anaphase plates of the first oöcyte division repeatedly give the count of 21 chromosomes. Figs. 11 and 12 show two daughter plates found superimposed within a single section and each gives the count of 21 chromosomes including the microsome. Such an observation of the dividing microsome, together with its constant behavior as a member of the X-group in the spermatocyte cells, gives the conviction that this minute body is in reality a chromosome. The second oöcyte plates (Fig. 13) again reveal the expected count of 21 chromosomes of which one is the microsome. Side views of both oöcyte anaphases show a clean separation of daughter plates with no sign of lagging chromosomes, so conspicuous in the first spermatocyte division.

These results demonstrate that in *Ascaris incurva* there are formed two classes of spermatozoa, one bearing 21 chromosomes, the other 14 chromosomes; and they indicate that fertilization of the egg carrying 21 chromosomes by a spermatozoön of the first class gives rise to the females which have 42 chromosomes and by one of the second class to the males which have 35 chromosomes. This cycle of the chromosomes may be summarized in the following formulae in which the autosomes are designated as A and the sex chromosomes as X and Y.

| Spermatozoa of Two Classes. | | Egg. | Gamete. |
|---|---|---|---|
| 13A + 8X | + | 13A + 8X = 26A + 16X = 42 (female). | |
| 13A + Y | + | 13A + 8X = 26A + 8X + Y = 35 (male). | |

I would like to express my thanks to Dr. Edwin Linton for advice and aid in obtaining material and to Dr. E. B. Wilson at

whose suggestion and under whose guidance the work has been undertaken.

COLUMBIA UNIVERSITY,
May, 1914.

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## EXPLANATION OF PLATE.

All figures are drawn with a Zeiss 1.5 mm. apochromatic objective, a no. 18 compensating ocular and projected with camera-lucida to table level. Figures as here reproduced give a magnification of 3,000 diameters. Figs. 1, 2, 3, 7, 8, 11, 12, 13 are from material fixed in Gilson-Carnoy's fluid and Figs. 4, 5, 6, 9, 10 from material fixed in strong Flemming's fluid.

FIG. 1. Spermatogonial metaphase.

FIG. 2. First spermatocyte metaphase showing the XY group centrally located and surrounded by 13 autosomes.

FIG. 3. First spermatocyte metaphase. An optical section of a side view showing the Y-chromosome opposite the end of the long X-chromosome, six other X-elements and three pairs of dividing autosomes.

FIGS. 4, 5, 6. First spermatocyte anaphase. Figures are from one spindle; Fig. 4 showing upper ring of 13 autosomes and Y, Fig. 6 showing lower plate of 13 autosomes and gap opposite position of Y in Fig. 4, Fig. 5 showing the intervening X-element of 8 chromosomes.

FIG. 7. First spermatocyte anaphase. A side view of late stage showing daughter plates of autosomes and X-group viewed edgewise.

FIG. 8. First spermatogonial anaphase. Late stage showing 5 autosomes from upper plate, 3 from lower and the X-group of 8 chromosomes.

FIG. 9. Second spermatocyte metaphase showing 14 chromosomes.

FIG. 10. Second spermatocyte metaphase showing 21 chromosomes.

FIGS. 11, 12. First oocyte anaphase. Daughter plates from one spindle and each showing 21 chromosomes.

FIG. 13. Second oocyte metaphase showing 21 chromosomes.

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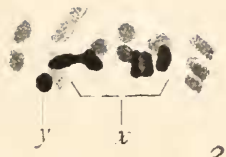


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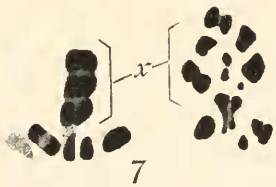


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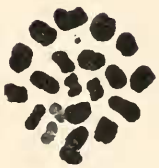

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# LINKAGE OF THE FACTOR FOR BIFID WING. THE BIFID WING AND OTHER SEX-LINKED FACTORS IN DROSOPHILA.

ROBERT CHAMBERS, Jr.

The experiments described in the following pages were made primarily in order to test whether crossing-over of factors has any subsequent effect on the linkage relations of the factors involved. For instance, if a red eyed fly with bifid wings is crossed to a white eyed fly with normal wings there will appear in $F_2$ the non-crossover classes, red bifid and white normal, and also some crossovers white bifid and red normal. These cross-overs (white bifid) were then used to determine whether the same linkage values would reappear in their grandchildren; in other words, whether a crossover in a particular place predisposes to more frequent crossing-over in the same place.

I wish to take this opportunity of acknowledging my indebtedness to Professor Morgan and to Mr. Bridges for their advice and suggestions.

The term "bifid" was given to a type of wing in which the second inner vein fails to reach the margin, often producing a bifid or forked wing.[1] The forked condition is variable. A constant feature, however, which the forked condition frequently accompanies, is a fusing of the wing veins at the base of the wing. Flies possessing this characteristic cannot fly.

The following abbreviations are used in the tables to denote the factors taken account of in the flies studied:

For eye color—R = red, V = vermilion, W = white;

For body color—Gr = Gray, Y = yellow;

For wing shape—L = long, Bf = bifid;

X = factor to which sex linked factors are linked;

o = gamete not possessing X-factor.

The factors R, V, Gr and Bf are in association with the X-factor which is duplex in the female and simplex in the male.

[1] Morgan, *Science*, Vol. 35, March 12, 1912.

For the sake of simplicity these factors will be considered in this paper as single units.

## Experiment I.

A long-winged vermilion eyed female was mated with a bifid winged red eyed male. The results to be expected in the $F^1$ and $F^2$ generations are shown in Table I.

### Table I.

To Illustrate Experiment I.

| | | |
|---|---|---|
| $P^1$ | LVX − LVX = LV ♀<br>$P^1$ Eggs LVX | BfRX − o = BfR ♂<br>Sperm BfRX, o |
| $F^1$ | LVX − BfRX = LR ♀ | LVX − o = LV ♂ |
| | $F_1$ Eggs { (1) LVX, (2) BfRX, (3) LRX, (4) BfVX, | Sperm { LVX, o |
| $F^2$ | LVX − LVX<br>BfVX − LVX } = LV ♀ | LVX − o = LV ♂<br>BfRX − o = BfR ♂ |
| | BfRX − LVX<br>LRX − LVX } = LR ♀ | LRX − o = LR ♂<br>BfVX − o = BfV ♂ |

The $F^1$ generation consisted of LR females and LV males. The actual results of the $F^2$ generation are shown in Table II.

### Table II.

Results of Experiment I.

| $F^2$ | ♀ | | ♂ | | | | Total ♂♂ | Per Cent. of Crossovers. |
|---|---|---|---|---|---|---|---|---|
| | LR | LV | BfR | LV | BfV | LR | | |
| | 273 | 256 | 123 | 144 | 50 | 62 | 379 | 30 |

All the combinations expected are realized. Owing, however, to the coupling in pairs of the $P^1$ factors, the numbers of males in the two classes consisting of the combinations LR and BfV are fewer than those of the other two classes. That they appear at all is evidence of the incompleteness of the linkage, the new combinations being due to a rearrangement or crossing over of factors within the germ cells of the $F^1$ flies. The strength of linkage may be determined by that percentage of the total which are crossovers, in this case 112/379 or approximately 30 per cent.

On mating the $F^2$ males possessing the new combination BfV

with normal red eyed long winged flies (LR) we obtain LR male and female offspring. When these are interbred we obtain the $F^2$ results shown in Table III.

## Table III.

Results of Experiment I., Crossover.

| $F^2$ | ♀ | ♂ | | | | Total ♂♂ | Per Cent. of Crossovers. |
|---|---|---|---|---|---|---|---|
| | LR | BfV | LR | LV | BfR | | |
| | 498 | 80 | 123 | 71 | 53 | 327 | 38 |

These results even with comparatively small numbers show that the new combination BfV produced by a crossing over of factors possesses approximately the same strength of coupling as did the original.

## Experiment II.

In the reciprocal cross, BfR females with LV males, the offspring consist of LR females and BfR males. In the second generation the four classes of males occur in the same proportionate numbers as those in Experiment I.

The results to be expected are shown in Table IV.

## Table IV.

To Illustrate Experiment II.

| | | |
|---|---|---|
| $P^1$ | BfRX − BfRX = BfR♀ | LVX − o = LV♂ |
| | $P^1$ Eggs BfRX | Sperm LVX, o |
| $F^1$ | BfRX − LVX = LR♀ | BfRX − o = BfR♂ |
| | $F_1$ Eggs { (1) BfRX, (2) LVX, (3) BfVX, (4) LRX, | Sperm { BfRX, o |
| $F^2$ | BfRX − BfRX, BfVX − BfRX } = BfR♂ | BfRX − o = BfR♂<br>LVX − o = LV♂ |
| | LVX − BfRX, LRX − BfRX } = LR♀ | BfVX − o = BfV♂<br>LRX − o = LR♂ |

The actual results of the experiment are shown in Table V.

## Table V.

Results of Experiment II.

| $F^2$ | ♀ | | ♂ | | | | Total ♂♂. | Per Cent. of Crossovers. |
|---|---|---|---|---|---|---|---|---|
| | LR | BfR | LV | BfR | LR | BfV | | |
| | 103 | 108 | 57 | 85 | 31 | 33 | 206 | 31 |

## Experiment III.

Experiment III. confirms the results of Experiment I. LV females were crossed with BfR males. The $F^1$ females, all of which possess the gametic constitution, LVX–BfRX, (see Table I.), instead of being allowed to breed with their $F^1$ brothers, were mated with normal wild males possessing the gametic constitution RLX–o. The results are shown in Table VI. Although the cultures show a rather wide range of fluctuation it is doubtful if any of these are significantly different from 32, which is the value given by the total.

### Table VI.

Results of Experiment III.

LV ♀ × BfR ♂

$F^1$ LR ♀ ♀ and LV ♂ ♂

LR ♀ of $F^1$ × pure LR ♂

| $F^2$ | | ♀ | | ♂ | | | | |
|---|---|---|---|---|---|---|---|---|
| Bottle No. | Transfer. | LR | | LV | BfR | LR | BfV | Per Cent. of Crossovers. |
| 1 | a | 185 | | 70 | 44 | 18 | 13 | |
| | b | 120 | | 37 | 33 | 18 | 15 | |
| | c | 72 | | 12 | 24 | 8 | 12 | |
| | | | 377 | 119 | 101 | 44 | 40 | 28 |
| 2 | a | 133 | | 40 | 39 | 20 | 16 | |
| | b | 93 | | 34 | 26 | 18 | 11 | |
| | | | 226 | 74 | 64 | 38 | 27 | 32 |
| 3 | a | 150 | | 50 | 50 | 22 | 18 | |
| | b | 97 | | 33 | 31 | 14 | 13 | |
| | | | 247 | 83 | 81 | 36 | 31 | 29 |
| 4 | a | 156 | | 50 | 39 | 19 | 17 | |
| | b | 105 | | 32 | 30 | 22 | 13 | |
| | | | 261 | 82 | 69 | 41 | 30 | 32 |
| 5 | a | 118 | | 26 | 26 | 19 | 13 | |
| | b | 94 | | 21 | 21 | 15 | 16 | |
| | — | | 212 | 47 | 47 | 34 | 29 | 40 |
| 6 | — | | 155 | 71 | 27 | 16 | 15 | 24 |
| 7 | — | | 181 | 58 | 42 | 21 | 22 | 30 |
| 8 | — | | 199 | 51 | 58 | 30 | 28 | 35 |
| 9 | — | | 112 | 21 | 16 | 15 | 9 | 39 |
| Totals | | | 1970 | 606 | 485 | 275 | 231 | 32 |

## Experiment IV.

LY females were mated with BfGr males. The $F^1$ generation consisted of LGr females and LY males. Interbreeding these

gave rise to the four possible classes of males as shown in Table VII.

## Table VII.

To Illustrate Experiment IV.

| | | |
|---|---|---|
| $P^1$ | LYX − LYX = LY♀ | BfGrX − o = BfGr♂ |
| | Gametes LYX | BfGrX, o |
| $F^1$ | LYX − BfGrX = LGr♀ | LYX − o = LY♂ |
| | $F_1$ Eggs { (1) LYX, (2) BfGrX, (3) LGrX, (4) BfYX | Sperm { LYX, o |
| $F^2$ | LYX − LYX, BfYX − LYX } = LY♀ | LYX − o = LY♂, BfGrX − o = BfGr♂ |
| | LGrX − LYX, BrGrX − LYX } = LGr♀ | LGrX − o = LGr♂, BfYX − o = BfY♂ |

The actual results of the experiment are shown in Table VIII.

## Table VIII.

Results of Experiment IV.

$P^1$ LY♀ × BfGr♂

$F^1$ LGr♀ × LY♂

| $F^2$ | ♀ | | ♂ | | | | Per Cent. of Crossovers. |
|---|---|---|---|---|---|---|---|
| Bottle No. | LGr | LY | BfGr | LY | BfY | LGr | |
| 1 | 69 | 29 | 46 | 41 | 0 | 7 | |
| 2 | 53 | 43 | 34 | 23 | 1 | 1 | |
| 3 | 70 | 59 | 57 | 46 | 5 | 1 | |
| 4 | 93 | 71 | 77 | 76 | 2 | 4 | |
| Totals | 285 | 202 | 214 | 186 | 8 | 13 | 5 |

Table VIII. shows that the linkage of the factors for bifid and yellow is stronger than for bifid and vermilion studied in Experiment I., the per cent. of crossovers being 5.

On mating males of the crossover class BfY with pure LR females we find in the $F^2$ generation (Table IX.) that the new combination persists in the same percentage as did the original combinations depicted in Table VIII. These results are in harmony with those obtained in Experiment I.

## Table IX.

Results of Experiment IV. The Reciprocal Cross.

| | |
|---|---|
| $P^1$ | BfGr ♀ × LY ♂ |
| $F^1$ | LR ♀ × LR ♂ |

| $F^2$ | ♀ | ♂ | | | | Per Cent. of Crossovers. |
|---|---|---|---|---|---|---|
| Bottle No. | LGr | LGr | BfY | LY | BfGr | |
| 5 | 126 | 67 | 53 | 3 | 5 | |
| 6 | 237 | 110 | 99 | 7 | 3 | |
| 7 | 102 | 50 | 37 | 2 | 1 | |
| 8 | 174 | 60 | 59 | 2 | 2 | |
| Total | 649 | 287 | 248 | 14 | 11 | 4.5 |

## Experiment V.

This experiment is a repetition, during the following summer, of Experiment IV. The results of both reciprocal crosses are given in Tables X. and XI.

In order to secure large numbers the parent flies were transferred to fresh bottles every ten days. The transfers are indicated in the second column.

## Table X.

Results of Experiment V. Repetition of Experiment IV. (Cf. Table VIII.)

| $F^2$ | | ♀ | | | ♂ | | | | Per Cent. of Crossovers. |
|---|---|---|---|---|---|---|---|---|---|
| Bottle No. | Transfer. | LGr | | LY | BfGr | LY | BfY | LGr | |
| 1 | a | 27 | | 26 | 35 | 20 | 4 | 3 | |
| | b | 96 | | 84 | 82 | 81 | 1 | 1 | |
| | | | 123 | 110 | 117 | 101 | 5 | 4 | |
| 2 | a | 54 | | 51 | 48 | 42 | 4 | 1 | |
| | b | 45 | | 31 | 33 | 26 | 4 | 3 | |
| | c | 61 | | 49 | 45 | 36 | 1 | 4 | |
| | | | 160 | 131 | 126 | 104 | 9 | 8 | |
| 3 | a | 113 | | 113 | 93 | 79 | 10 | 4 | |
| | b | 167 | | 150 | 149 | 114 | 8 | 7 | |
| | | | 280 | 263 | 242 | 193 | 18 | 11 | |
| 4 | a | 19 | | 32 | 18 | 16 | 1 | 1 | |
| | b | 78 | | 66 | 75 | 73 | 7 | 4 | |
| | c | 232 | | 69 | 198 | 64 | 6 | 9 | |
| | d | 97 | | 12 | 77 | 11 | — | 7 | |
| | | | 426 | 179 | 368 | 164 | 14 | 21 | |
| Totals | | | 989 | 683 | 853 | 562 | 46 | 44 | 6 |

The results of the reciprocal cross are shown in Table XI.

## Table XI.

Results of Experiment V. The Reciprocal Cross. (Cf. Table IX.)

| $F^2$ | | ♀ | | ♂ | | | | Per Cent. of Cross-overs. |
|---|---|---|---|---|---|---|---|---|
| Bottle No. | Transfer. | LGr | BfGr | LY | BfGr | LGr | BfY | |
| 5 | a | 33 | 38 | 24 | 30 | 2 | 3 | |
| | b | 88 | 51 | 77 | 73 | 6 | 5 | |
| | c | 59 | 40 | 36 | 33 | 1 | 0 | |
| | d | 55 | 37 | 43 | 46 | 5 | 5 | |
| | | 235 | 166 | 180 | 182 | 14 | 13 | |
| 6 | | 194 | 146 | 111 | 148 | 16 | 5 | |
| 7 | | 75 | 39 | 50 | 53 | 0 | 4 | |
| 8 | | 121 | 114 | 78 | 82 | 4 | 4 | |
| 9 | | 104 | 96 | 40 | 69 | 4 | 4 | |
| Total | | 729 | 561 | 459 | 534 | 38 | 30 | 6 |

The percentage of crossovers is the same as that shown in Table X. and slightly larger than that in the corresponding experiment in Table IX.

### Experiment VI.

LW females were mated with BfR males.

The LR females and LW males of the $F^1$ generation were allowed to interbreed. Table XII gives the numbers produced in the resulting $F^2$ generation. The percentage of crossovers is approximately 8.

## Table XII.

Results of Experiment VI.

LW ♀ × BfR ♂

$F^1$ LR ♀ × LW ♂

| $F^2$ | ♀ | | ♂ | | | | Per Cent. of Crossovers. |
|---|---|---|---|---|---|---|---|
| Bottle No. | LR | LW | LW | BfR | LR | BfW | |
| 1 | 218 | 182 | 194 | 181 | 16 | 12 | 7 |
| 2 | 94 | 85 | 100 | 103 | 15 | 15 | 13 |
| 3 | 115 | 86 | 69 | 77 | 2 | 5 | 5 |
| Total | 427 | 353 | 363 | 361 | 33 | 32 | 8 |

The strength of coupling of the new combinations in the crossovers was determined by mating the crossover BfW males with pure LR ♂. Table XIII. gives the results of this mating.

TABLE XIII.

RESULTS OF EXPERIMENT VI.- CROSSOVERS.

LR ♀ × BfW ♂

| $F^2$ | LR ♀ × LR ♂ | | | | | |
|---|---|---|---|---|---|---|
| $F^2$ | ♀ | ♂ | | | | Per Cent. of Crossovers. |
| Bottle No. | LR | LR | BfW | LW | BfR | |
| 1 | 329 | 144 | 111 | 11 | 12 | 7 |
| 2 | 394 | 166 | 145 | 15 | 15 | 9 |
| 3 | 193 | 96 | 64 | 5 | 7 | 7 |
| 4 | 438 | 173 | 147 | 21 | 18 | 11 |
| 5 | 152 | 74 | 51 | 7 | 8 | 11 |
| 6 | 301 | 106 | 58 | 6 | 6 | 7 |
| Total | 1807 | 759 | 576 | 65 | 66 | 9 |

The crossing back of the crossovers occurs in approximately the same percentage as did the crossovers to the original combinations in Experiment VII.

The results here harmonize with those of Experiment I. We may legitimately infer that the large classes in the $F^2$ generation are those with the combinations occurring in the grandparents no matter whether the grandparents have acquired those factors early or late in their phylogenesis.

## EXPERIMENT VII.

The results of the reciprocal cross, BfR females by LW males is shown in Table XIV.

TABLE XIV.

RESULTS OF EXPERIMENT VII. RECIPROCAL OF EXPERIMENT VI.

BfR ♀ × LW ♂

| $F^1$ | LR ♀ × BfR ♂ | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $F^2$ | | ♀ | | ♂ | | | | Per Cent. of Crossovers. |
| Bottle No. | Transfer. | LR | BfR | LW | BfR | LR | BfW | |
| 1 | a | 161 | 99 | 108 | 105 | 6 | 7 | |
| | b | 135 | 77 | 100 | 87 | 4 | 6 | |
| | c | 18 | 10 | 17 | 14 | 0 | 1 | |
| | | 314 | 186 | 225 | 206 | 13 | 14 | 6 |
| 2 | | 76 | 54 | 85 | 65 | 3 | 4 | 4 |
| 3 | | 117 | 91 | 86 | 83 | 6 | 4 | 6 |
| 4 | | 166 | 136 | 130 | 146 | 5 | 3 | 3 |
| 5 | | 157 | 119 | 113 | 129 | 3 | 2 | 2 |
| 6 | | 174 | 142 | 113 | 120 | 5 | 3 | 3 |
| 7 | | 111 | 80 | 81 | 83 | 2 | 3 | 3 |
| 8 | | 120 | 75 | 64 | 94 | 3 | 7 | 6 |
| 9 | | 143 | 117 | 107 | 107 | 5 | 4 | 4 |
| Total | | 1378 | 1000 | 1004 | 1033 | 45 | 44 | 4 |

The number of crossovers is far too small to give the same ratio found in Experiment VI. Here the percentage is only 4.

## Experiment VIII.

This is a modification of Experiment VI. LR females were mated with BfW males. The $F^1$ generation consisted of LR males and females. The LR ♀ were removed from their brothers and crossed with pure BfW males. This was done in order to secure four possible classes, in the $F^2$ generation, not only of males but also of females thus rendering the female counts also available for study.

Table XV. shows the gametic constitution of the flies used and the combinations expected in the $F^1$ and $F^2$ generations.

### Table XV.

To Illustrate Experiment VIII.

| | | |
|---|---|---|
| $P^1$ | LRX − LRX = LR ♀ | BfWX − o = BfR ♂ |
| | Gametes LRX | BfWX, o |
| $F^1$ | LRX − BfWX = LR ♀ | LRX − o = LR ♂ |
| | LR ♀ of $F^1$ × BfW ♂ (from stock) | |
| | $F_1$ Eggs { (1) LRX, (2) BfWX, (3) LWX, (4) BfRX | Sperm { BfWX, o |
| $F^2$ | LRX − BfWX = LR ♀ | LRX − o = LR ♂ |
| | BfWX − BfWX = BfW ♀ | BfWX − o = BfW ♂ |
| | LWX − BfWX = LW ♀ | LWX − o = LW ♂ |
| | BfRX − BfWX = BfR ♀ | BfRX − o = BfR ♂ |

In Table XVI. are given the actual results of the experiment. Judging from the total numbers of the $F^2$ flies the break in the coupling occurs in the ratio of 5 per cent. in both females and males. Only the normal range of variations from this are apparent when the numbers from individual bottles are considered.[1]

[1] In the previous experiments 4–6 females were placed with as many males in the same bottle. For Experiments VIII. and IX. one female with 2–3 males was placed in a bottle. She and the males were transferred every 6–8 days. Some of the females lasted for six weeks by which time they were fairly exhausted of eggs.

## TABLE XVI.

### RESULTS OF EXPERIMENT VIII.

LR ♀ × BfW ♂

$F^1$ LR ♂

LR ♀ of $F^1$ × BfW ♂ (from stock bottle).

| $F^2$ Bottle No. | Transfer. | ♀ LR | | BfW | | LW | BfR | Per Cent. of Crossovers. | ♂ LR | | BfW | | LW | BfR | Per Cent. of Crossovers. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | a | 45 | | 30 | | 3 | 2 | | 36 | | 35 | | 3 | 1 | |
| | b | 39 | | 26 | | 4 | 1 | | 56 | | 26 | | 1 | 4 | |
| | c | 83 | | 83 | | 5 | 7 | | 66 | | 92 | | 3 | 5 | |
| | d | 63 | | 53 | | 1 | 6 | | 61 | | 76 | | 7 | 4 | |
| | e | 15 | | 12 | | 0 | 0 | | 17 | | 13 | | 2 | 1 | |
| | f | 2 | | 1 | | 0 | 0 | | 1 | | 0 | | 0 | 0 | |
| | | | 247 | | 205 | 13 | 16 | 6 | | 237 | | 242 | 16 | 15 | 6 |
| 2 | a | 40 | | 19 | | 0 | 2 | | 35 | | 34 | | 0 | 2 | |
| | b | 45 | | 55 | | 3 | 1 | | 50 | | 44 | | 3 | 3 | |
| | c | 66 | | 75 | | 4 | 1 | | 67 | | 75 | | 2 | 3 | |
| | d | 51 | | 46 | | 5 | 4 | | 48 | | 39 | | 3 | 2 | |
| | e | 33 | | 39 | | 2 | 1 | | 32 | | 34 | | 4 | 2 | |
| | | | 235 | | 234 | 14 | 9 | 5 | | 232 | | 226 | 12 | 12 | 5 |
| 3 | a | 52 | | 35 | | 4 | 1 | | 29 | | 47 | | 4 | 1 | |
| | b | 20 | | 24 | | 3 | 0 | | 29 | | 23 | | 1 | 1 | |
| | c | 34 | | 28 | | 4 | 1 | | 44 | | 25 | | 0 | 3 | |
| | d | 29 | | 22 | | 2 | 1 | | 23 | | 29 | | 0 | 1 | |
| | e | 10 | | 11 | | 3 | 0 | | 8 | | 12 | | 2 | 1 | |
| | | | 145 | | 120 | 16 | 3 | 7 | | 133 | | 136 | 7 | 7 | 5 |
| 4 | a | 88 | | 90 | | 6 | 3 | | 94 | | 102 | | 10 | 1 | |
| | b | 105 | | 101 | | 8 | 4 | | 90 | | 122 | | 4 | 5 | |
| | c | 54 | | 38 | | 0 | 2 | | 59 | | 49 | | 3 | 3 | |
| | d | 28 | | 37 | | 7 | 3 | | 32 | | 33 | | 2 | 3 | |
| | e | 23 | | 12 | | 1 | 1 | | 17 | | 14 | | 2 | 1 | |
| | | | 298 | | 278 | 22 | 13 | 6 | | 292 | | 320 | 21 | 13 | 5 |
| 5 | a | 57 | | 70 | | 4 | 1 | | 59 | | 71 | | 1 | 3 | |
| | b | 38 | | 33 | | 4 | 1 | | 38 | | 41 | | 0 | 2 | |
| | c | 24 | | 33 | | 1 | 0 | | 23 | | 24 | | 0 | 2 | |
| | d | 19 | | 18 | | 1 | 0 | | 14 | | 14 | | 1 | 2 | |
| | | | 138 | | 154 | 10 | 2 | 4 | | 134 | | 150 | 2 | 9 | 4 |
| 6 | | | 103 | | 73 | 7 | 6 | 7 | | 97 | | 79 | 6 | 4 | 5 |
| 7 | | | 38 | | 30 | 0 | 2 | 3 | | 56 | | 45 | 2 | 3 | 5 |
| 8 | | | 120 | | 93 | 8 | 7 | 7 | | 90 | | 125 | 6 | 4 | 4 |
| 9 | | | 209 | | 190 | 5 | 5 | 2 | | 185 | | 169 | 13 | 14 | 7 |
| 10 | | | 88 | | 66 | 5 | 1 | 4 | | 61 | | 79 | 7 | 3 | 7 |
| 11 | | | 63 | | 60 | 5 | 3 | 6 | | 95 | | 77 | 5 | 7 | 7 |
| 12 | | | 162 | | 143 | 8 | 5 | 4 | | 149 | | 129 | 9 | 11 | 7 |
| 13 | | | 162 | | 141 | 8 | 7 | 5 | | 170 | | 159 | 7 | 8 | 4 |
| 14 | | | 156 | | 138 | 10 | 6 | 5 | | 196 | | 157 | 12 | 7 | 5 |
| 15 | | | 139 | | 116 | 5 | 4 | 3 | | 97 | | 111 | 6 | 4 | 5 |
| 16 | | | 181 | | 155 | 13 | 5 | 7 | | 171 | | 154 | 8 | 9 | 5 |
| Total | | | 2322 | | 2053 | 141 | 89 | 5 | | 2246 | | 2229 | 130 | 119 | 5 |

## Experiment IX.

For this experiment both males and females with crossed over factors were used. These were taken from the $F^2$ generation produced in Experiment VIII. On looking over Table XV. it will be noticed that the $F^2$ BfR females possess the gametic constitution of BfRX–BfWX. Both of the combinations BfR and BfW have been produced in the normal percentage by a crossing over in the $F^1$ generation of factors originally coupled in the grandparents.

The $F^2$ LW males have been similarly produced, their factors L and W being crossovers.

All combinations, therefore, introduced into the $F^1$ generation have been produced by a rearrangement of the original combinations owing to crossing over.

Table XVII. shows the expected results of such a crossing.

### Table XVII.

To Illustrate Experiment X. (Crossovers.)

| | | |
|---|---|---|
| $P^1$ | BfRX – BfWX = BfR ♀ | LWX – o = LW ♂ |
| | Gametes BfRX, BfWX | LWX, o |
| $F^1$ | BfRX – LWX = LR ♀ | BfRX – o = BfR ♂ |
| | BfWX – LWX = LW ♀ | BfWX – o = BfW ♂ |
| | LR of $F^1$ × BfW of $F^1$ | |
| | $F_1$ Eggs { (1) BfRX, (2) LWX, (3) BfWX, (4) LRX | Sperm { BfWX, o |
| $F^2$ | BfRX – BfWX = BfR ♀ | BfRX – o = BfR ♂ |
| | LWX – BfWX = LW ♀ | LWX – o = LW ♂ |
| | BfWX – BfWX = BfW ♀ | BfWX – o = BfW ♂ |
| | LRX – BfWX = LR ♀ | LRX – o = LR ♂ |

In spite of the conditions of the experiment the combinations introduced into the $F^1$ generation persist with a strength of linkage approximately equal to that existing among the original combinations.

Table XVIII. gives the actual results of the experiment.

## TABLE XVIII.

RESULTS OF EXPERIMENT X.

BfR ♀ × LW ♂ (Both crossovers from $F^2$ of Table 16).

$F^1$ LR and LW ♀ ♀, BfR and BfW ♂ ♂

LR ♀ of $F^1$ × BfW ♂ (from stock bottle)

| $F^2$ | | ♀ | | | | | | | ♂ | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bottle No. | Transfer. | BfR | | LW | | BfW | LR | | BfR | | LW | | BfW | LR | |
| 17 | a | 89 | | 62 | | 1 | 3 | | 78 | | 64 | | 5 | 2 | |
| | b | 46 | | 39 | | 1 | 0 | | 41 | | 46 | | 0 | 1 | |
| | c | 33 | | 31 | | 0 | 2 | | 26 | | 32 | | 3 | 3 | |
| | d | 39 | | 46 | | 2 | 3 | | 33 | | 32 | | 2 | 1 | |
| | e | 28 | | 28 | | 0 | 5 | | 23 | | 29 | | 4 | 3 | |
| | f | 2 | | 0 | | 0 | 0 | | 2 | | 4 | | 0 | 0 | |
| | | | 237 | | 206 | 3 | 13 | 3 | | 203 | | 207 | 14 | 10 | 6 |
| 18 | a | 32 | | 37 | | 3 | 3 | | 38 | | 30 | | 3 | 0 | |
| | b | 56 | | 41 | | 3 | 2 | | 56 | | 48 | | 5 | 2 | |
| | c | 21 | | 22 | | 3 | 0 | | 21 | | 15 | | 2 | 0 | |
| | d | 31 | | 29 | | 2 | 3 | | 32 | | 24 | | 5 | 4 | |
| | e | 27 | | 31 | | 0 | 3 | | 38 | | 28 | | 3 | 2 | |
| | f | 6 | | 5 | | 1 | 0 | | 4 | | 5 | | 0 | 0 | |
| | | | 173 | | 165 | 12 | 11 | 6 | | 189 | | 150 | 18 | 8 | 7 |
| 19 | a | 49 | | 57 | | 2 | 5 | | 67 | | 62 | | 3 | 1 | |
| | b | 30 | | 30 | | 2 | 3 | | 26 | | 29 | | 2 | 2 | |
| | c | 28 | | 23 | | 2 | 3 | | 22 | | 18 | | 3 | 1 | |
| | d | 37 | | 43 | | 0 | 4 | | 38 | | 32 | | 5 | 1 | |
| | e | 16 | | 14 | | 3 | 0 | | 15 | | 22 | | 1 | 3 | |
| | f | 5 | | 1 | | 0 | 0 | | 4 | | 4 | | 0 | 0 | |
| | | | 165 | | 168 | 9 | 15 | 7 | | 172 | | 167 | 14 | 8 | 7 |
| 20 | a | 65 | | 65 | | 0 | 5 | | 63 | | 59 | | 8 | 1 | |
| | b | 42 | | 31 | | 1 | 1 | | 27 | | 47 | | 5 | 2 | |
| | c | 34 | | 46 | | 2 | 1 | | 38 | | 40 | | 1 | 1 | |
| | d | 54 | | 31 | | 6 | 1 | | 48 | | 29 | | 3 | 1 | |
| | e | 4 | | 3 | | 0 | 1 | | 9 | | 3 | | 0 | 1 | |
| | | | 199 | | 176 | 9 | 9 | 5 | | 185 | | 178 | 17 | 6 | 6 |
| 21 | a | 19 | | 38 | | 1 | 2 | | 37 | | 39 | | 3 | 0 | |
| | b | 31 | | 37 | | 2 | 2 | | 37 | | 51 | | 4 | 2 | |
| | c | 52 | | 48 | | 4 | 1 | | 36 | | 38 | | 2 | 2 | |
| | d | 65 | | 53 | | 3 | 2 | | 60 | | 53 | | 4 | 5 | |
| | e | 19 | | 13 | | 0 | 1 | | 22 | | 7 | | 1 | 0 | |
| | | | 186 | | 189 | 10 | 8 | 5 | | 192 | | 188 | 14 | 9 | 6 |
| 22 | | | 159 | | 134 | 5 | 6 | 4 | | 185 | | 135 | 9 | 6 | 4 |
| 23 | | | 142 | | 115 | 4 | 2 | 2 | | 135 | | 106 | 4 | 5 | 4 |
| 24 | | | 16 | | 13 | 0 | 0 | | | 10 | | 9 | 0 | 0 | |
| 25 | | | 62 | | 35 | 4 | 1 | 5 | | 50 | | 37 | 1 | 0 | 1 |
| 26 | | | 44 | | 31 | 2 | 2 | 5 | | 30 | | 36 | 0 | 5 | 6 |
| 27 | | | 64 | | 56 | 3 | 2 | 4 | | 64 | | 52 | 0 | 5 | 4 |
| 28 | | | 92 | | 89 | 1 | 8 | 5 | | 80 | | 89 | 3 | 6 | 5 |
| 29 | | | 97 | | 92 | 4 | 6 | 5 | | 100 | | 70 | 5 | 10 | 8 |
| 30 | | | 48 | | 52 | 3 | 0 | 3 | | 55 | | 62 | 2 | 1 | 3 |
| 31 | | | 98 | | 104 | 4 | 8 | 6 | | 99 | | 85 | 6 | 13 | 9 |
| Total | | | 1782 | | 1625 | 73 | 91 | 5 | | 1749 | | 1571 | 107 | 92 | 6 |

On examining the numbers from the individual bottles only the normal fluctuation in the strength of linkage is noticeable.

Summary.

The strength of linkage between the factor for bifid wing and the factor for vermilion eye is approximately the same (viz. 32 units) in the original cross in its reciprocal, and in the $F_2$ from the crossovers of the original. (See Experiments I., II. and III., Tables II., III., V. and VI.)

The strength of linkage between yellow body color and bifid wing is constant (viz. 5 units) in the original and in the $F_2$ from the crossovers (See Experiment IV., Tables VII., VIII. and IX.).

A repetition of Experiment IV. showed a linkage value (viz. 6 units) not significantly different from that previously found. The linkage moreover is constant in the two reciprocal crosses of this experiment. (See Experiment V.; Tables X. and XI.)

The strength of linkage between white eyes and bifid wings is preserved in the crossovers (viz. original 8, crossover 9 units), but is different in the reciprocal (viz., 4). (See Experiments VI. and VII.; Tables XII., XIII. and XIV).

A modification of experiments VI. and VII. gave a strength of linkage (viz. 5 and 6), which approaches that of the reciprocal (viz., 4) but not the original linkage value (viz., 8). (See Experiments VIII. and IX.; Tables XVI. and XVIII.)

In all of the experiments of this paper the strength of linkage is apparently not changed by a previous crossing over between the factors in question.

The linkage value given by the females is the same as that given by the males of the same experiment. (See Tables XVI. and XVIII.)

Crossovers appear in the $F_2$ generation equally frequently among the first flies hatched as among those hatched last. That the factor for yellow body color has an effect on the viability of flies is evidenced from the deficient numbers of yellow flies in Tables VIII., X. and XI. A deficiency also occurs in flies with white eyes as compared with those possessing red eyes. (See Tables XIII., XVI. and XVIII.) A bifid wing factor, however, does not seem to have any such effect; the bifid winged flies comparing favorably with the long winged flies (see Table XVIII.).

University of Cincinnati,
May 6, 1914.

# NOTES ON REGRESSION IN A PURE LINE OF PLANT LICE.

H. E. EWING.

In a previous number of the BIOLOGICAL BULLETIN[1] the writer published the results of some selections made within a pure line of plant lice. These were made in an attempt to increase and to decrease the length of the third segments of the antennæ in comparison with that of the fourth segments. The species used was our common European grain aphis, *Aphis avenæ* Fab.; and the results obtained up to that time, which included the first ten generations, appeared to show that selections from extreme variants did not alter the mean as obtained for the line without selection. These results were in accord with the results obtained by other workers in pure lines.

But it also appeared that the mean of the offspring of the variants selected reverted not to the mean of the line, or strain, but that it would swing pendulum-like much beyond this mean only to be brought back to the former side of the mean-of-the-strain base line in the next generation.

Since the publication of this former paper I have tested further this aspect of regression by selecting from opposite extremes in alternating generations. These selections were begun in the ninth filial generation, and continued into the fifteenth, including in all seven generations. I will now give briefly the results of these selections.

An individual with the remarkably high index of 2.82 : 1 (*i. e.*, the third segments of the antennæ were two and eighty-two hundredths times as long as the fourth segments) was selected as the stem progenitor from among the individuals of the $F_8$ fraternity,—which fraternity has been mentioned in my previous paper. From this individual there were reared five wingless adults which gave a fraternal mean of 1.75 : 1, *i. e.*, .05 below the mean of the strain which was 1.80 : 1. From the $F_9$ fraternity,

[1] Vol. XXVI., No. 1, January, 1914.

$F_9$16 was selected for carrying on the strain. It had an index of 1.58 : 1, and gave a fraternity with a mean of 1.82 : 1. From this $F_{10}$ fraternity, $F_{10}$4 with an index of 2.11 : 1 was chosen. The mean for its offspring was 1.89 : 1. From this $F_{11}$ fraternity, $F_{11}$4, formula 1.78 : 1, was isolated. It gave the $F_{12}$ fraternity, with a mean of 1.84 : 1. $F_{12}$6, formula 1.98 : 1, gave the $F_{13}$ fraternity, with a mean of 1.85 : 1. $F_{13}$4, the next parent selected, had an index of 1.67 : 1, and gave the $F_{14}$ fraternity with a mean of 1.69 : 1. From the $F_{14}$ fraternity the last selection was made. $F_{14}$1, formula 1.70 : 1, was isolated, and gave the $F_{15}$ fraternity with 1.81 : 1 as a mean.

In each of these selections the individual with either the highest or the lowest antennal index was isolated in order to obtain the next generation of descendants. As has been stated, the index as obtained without selection for the mean of the pure line was 1.80 : 1. Thus it will be noticed that in four of the seven selections made, the offspring of the extreme variant gave a fraternal mean which showed a regression beyond the mean of the strain. In the case of the other three selections the regression of the fraternal mean did not reach the mean of the strain.

In order to observe more fully the effects of selection and the action of regression, I will give here in the order of their ratios the antennal formulæ of the eighteen parents thus far obtained in the first fifteen generations of the pure line[1] together with the indices representing the fraternal means of their offspring. They are as follows:

| Indices for Parents. | Indices for Fraternal Mean of Offspring. |
|---|---|
| 1.66 | 1.88 |
| 1.67 | 1.77 |
| 1.67 | 1.83 |
| 1.67 | 1.69 |
| 1.70 | 1.81 |
| 1.77 | 1.77 |
| 1.77 | 1.71 |
| 1.78 | 1.84 |
| 1.79 | 1.95 |
| 1.80 | 2.01 |
| 1.85 | 1.80 |
| 1.86 | 1.77 |

[1] Two of the parents which had antennal indices such as to suggest mutations or abnormalities are omitted.

| | |
|---|---|
| 1.86 | 1.85 |
| 1.88 | 1.80 |
| 1.89 | 1.93 |
| 1.98 | 1.85 |
| 2.08 | 1.66 |
| 2.11 | 1.89 |

If we now plot these results by using a scheme similar to that employed to illustrate Galton's law of regression we shall have the following graphical representation (Fig. 1) of the regression as found up to the fifteenth generation in this pure line of plant lice.

Here the heavy line represents the mean-of-the-strain base line, and is placed at 1.80. The lighter parallel lines above and

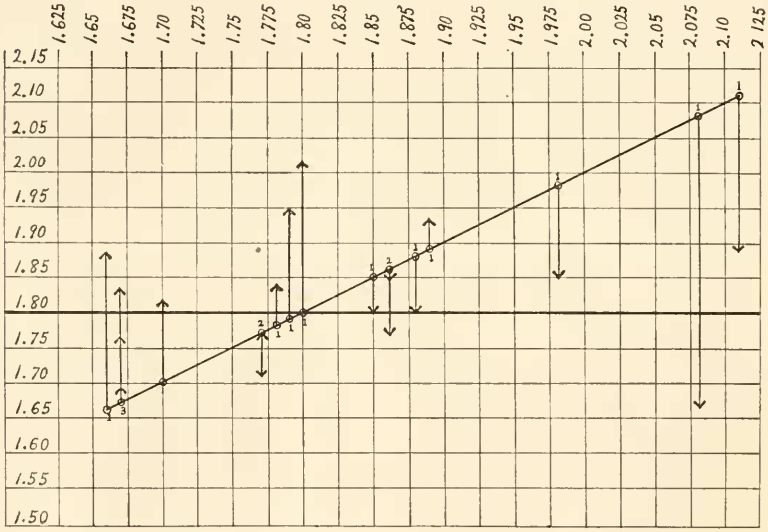


FIG. 1.—Diagram showing regression within a pure line of *Aphis avenæ* Fab. The heavy base line at 1.80 represents the position of the mean of the line, or strain. The small circles represent the positions of the parents according to the index of their third and fourth antennal segments; the small number placed by each circle shows the number of parents with the antennal index indicated by the circle; the arrow-heads represent the positions of the mean of the fraternities obtained from these various parents. The arrows show the amount of regression.

below this base line each represent an increase or decrease in the index ratio for the third and fourth antennal segments of five one-hundredths. Similarly one of the vertical lines represents the mean-of-the-strain base line, and is marked 1.80; and on

each side of it other parallel lines are laid off at equal distances. Each of these represents a difference of 0.025 in the index ratio for the antennal segments considered.

On this diagram small round circles have been placed representing the position of each of the eighteen parents according to their antennal index-ratio. These naturally form a straight line which is indicated. Now the mean index of the offspring of each parent is represented by an arrow-head, placed immediately above or below, as the case may be, the circle indicating the index of the parent. A line connecting these two points forms the arrow which indicates the actual amount of regression in each instance. In three instances we have more than one stem parent with the same index-ratio, hence in these cases the arrows showing regression are superimposed.

It is observed that in some instances the regression is not to the mean of the strain, and in other instances it is much beyond the mean of the strain; while in two instances there is no regression at all, but a deviation from the mean of the strain even greater than that that existed before in the parent.

In order to compute the average amount of regression we may arrange in parallel series the deviations as shown in the indices of the various parents from that of the mean of the strain, and the deviation shown by the average for the indices of their progenies. This is here done, the mean of the strain being given as zero and the deviation found in the various antennal ratios from this mean being given in hundredths, plus or minus, as the case may be.

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Deviations in indices of parents | − 14 | − 13 | − 13 | − 13 | − 10 | − 3 | − 3 | − 2 | − 1 | 0 | + 5 | + 6 | + 6 | + 8 | + 9 | + 18 | + 28 | + 31 |
| Average deviation found in offspring | + 8 | − 3 | + 3 | − 11 | + 1 | − 3 | − 9 | + 4 | + 15 | + 21 | 0 | − 3 | + 5 | 0 | + 13 | + 5 | − 14 | + 9 |

This relation of the regression can be expressed in the form of fractions by taking in each instance the difference in the deviation of the offspring from that of the parent, for the numerator; and the deviations of the parent itself in each instance as the denominator. These will then be:

+ 22/14, + 10/13, + 16/13, + 2/13, + 11/10, 0, − 6/3, + 6/2, + 16/1, + 5/5, + 9/6, + 1/6, + 8/8, − 4/9, + 13/18, + 42/28, + 22/31.

We may also express this series in the form of decimals, which will be as follows:

1.57, .76, 1.23, .15, 1.10, 0, − 2.00, 3.00, 16.00, 1.00, 1.50, .16, 1.00, − .44, .72, 1.50, .70.

These fractions added together and divided by their number should give us the average amount of regression. If the regression is according to Galton's law the decimal should be 0.333; if according to Johannsen's predictions, that is if the regression is complete, it should be 1.00. The figure which we actually obtain by adding these fractions and dividing by their number is 1.64. In other words, the regression is more than complete, or beyond the mean of the strain. However, it should be noted that the number of individuals included in this computation is too small to permit the results to be conclusive. Yet the results show that regression in a pure line of a parthenogenetic form does not follow Galton's law, also that there appears to be some justification in the contention made in a previous paper of mine, that regression under these conditions is somewhat pendulum-like, swinging beyond the mean of the strain, or line.

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# BIOLOGICAL BULLETIN

## REGENERATION OF *PLEUROTRICHA* AFTER MEROTOMY WITH REFERENCE ESPECIALLY TO THE NUMBER OF MICRONUCLEI AND THE OCCURRENCE OF UNINUCLEATE CELLS.[1]

J. H. HEWITT.

Lewin,[1] working with *Stylonychia*, has reported a series of experiments in which, after merotomy, he observed an increase in the number of micronuclei in the regenerated merozoa. It was thought to be of interest to repeat Lewin's experiments on another member of the hypotrichus group to determine if there might be any general application of the phenomenon he had observed. Pleurotricha was chosen for the experiments.

### Material.

The animals used were secured from a strain, the originator of which was isolated by Dr. George A. Baitsell in the biological laboratory of Yale University. The strain had been adapted to laboratory hay infusion media and was preserved as a stock culture in sterile test-tubes plugged with cotton. Such a culture tube of organisms was given me and from it subcultures were made to other tubes of fresh media and to fresh media in small glass capsules.

The hay infusion used as medium was prepared by placing about 10 grams of field hay in 200 c.c. of tap water in an Erlenmeyer flask and boiling it over a bunsen burner for a few minutes. This medium was made up but once and was used in the proportion of one drop of the infusion to five drops of tap water, this amount of fluid in a capsule serving as the medium for a single animal for twenty-four hours, or till it divided.

[1] From the Marine Biological Laboratory, Woods Hole, Mass.

## Method.

Merotomy was performed according to the method of Calkins. The animal selected was drawn up into a fine pointed pipet and placed on a clean glass slide under a Greenough's binocular microscope, eye-pieces 4 and objectives a. The medium was then drawn off till a drop of only sufficient size for the animal to swim in freely was left. With an ophthalmologist's iridectomy knife the animals were cut in parts. The point was ground off the knife and one edge ground to a semi-bellied shape. The blade was plunged into the drop with the animal and the posterior point of its edge allowed to rest on the surface of the slide. As the animal passed from one side to the other of the drop, or around the resting point of the knife edge, successive attempts were made by moving the knife handle up and down to cut the animal as it came directly in line with the edge of the knife.

The frangibility of the cell body afforded one of the first methods of securing fragments of infusoria for study. *Pleurotricha* appears to be very frangible. On one occasion, experiment 48, catching an animal on the surface of the media with a sudden and forcible blast of air from a fine pointed pipet the animal was broken in two. Simply drawing the animal rather forcibly in the pipet was sometimes sufficient to break it in two as in experiment 40. In a few of the experiments the merozoa were secured by drawing the animal selected into a fine pointed pipet and spurting it out forcibly on the side of the capsule.

Animals to be stained were isolated from the capsule with a fine pipet on a clean microscopical slide under a binocular microscope. They were killed and fixed in 5 per cent. glacial acetic acid in saturated mercuric chloride, stained by the Heidenhain iron hematoxylin method, and mounted in xylol balsam.

## The Effect of Merotomy on the Number of Micronuclei.

In Tables I. to V., inclusive, are recorded 27 experiments in which the regenerated merozoön was recovered, successfully stained, and mounted. The tables are divided according to the position of the cut. They also state the exact time before or after division, when it had been observed, the length of time after merotomy before the animal was killed, and the number of macronuclei and micronuclei found in the stained merozoön.

In experiments 31, 32, 33 and 57 the animals were killed from 38 minutes to 3 hours and 30 minutes after merotomy. All of these, except experiment 31, were mid-body cuts and it is reasonable to assume that the condition found is normal,—the macronucleus and the micronucleus having not yet divided, or being cells of the uninucleate variety which were also found in control

TABLE I.

ANTERIOR-END CUTS.

| Exp. No. | When Cut. | When Killed. | Number of Macronuclei, Number of Micronuclei. |
|---|---|---|---|
| 45 | — | 9 hrs. 53 min. after cut. | 2 macronuclei, 2 micronuclei |
| 105 | Before division | 27′ 25″ after cut. | 2 macronuclei, 2 micronuclei |

cultures of this animal. In experiment 57 the micronucleus is in mitosis and the cell appears normal.

In experiment 22, also a mid-body cut, 1 hour and 10 minutes after operation, there are two micronuclei in mitosis. The nucleus is single, swollen and enlarged, as if ready to divide. The most plausible explanation of the condition found here is that the cell originally had two macronuclei and three micronuclei, as was found in certain animals taken from culture.

TABLE II.

POSTERIOR-END CUTS.

| Exp. No. | When Cut. | When Killed. | Number of Macronuclei, Number of Micronuclei. |
|---|---|---|---|
| 4 | 1 hr. 7 min. after division. | 3 hrs. 18 min. after cut | 2 macronuclei, 2 micronuclei. |
| 15 | Before division | 6 min. after cut | 2 " 2 " |
| 27 | " | 30 " " " | 2 " 2 " |
| 33 | " | 38 " " " | 1 " 1 " |
| 34 | " | 1 hr. 10 min. after cut | 2 " 9 " |
| 39 | " | 8 hrs. 27 " " " | 2 " 2 " |
| 49 | " | 18 hrs. 7 " " " | 2 " 2 " |
| 106 | " | 27 hrs. 15 " " " | 2 " 2 " |
| 107 | " | 26 hrs. 55 " " " | 2 " 2 " |
| 109 | " | 22 hrs. 36 " " " | 2 " 2 " |

When merotomy was performed the animal was separated in two merozoa, one of which had a single macronucleus and a single micronucleus; the other merozoön which was saved and which regenerated had a single macronucleus and two micronuclei.

In experiment 34, a posterior-end cut, 1 hour and 10 minutes after operation, we have an abnormal and an interesting animal. The cell is pointed at both ends and broad in the middle, measuring 112 x 45 micra. The anterior macronucleus is situated

TABLE III.

MID-BODY CUTS.

| Exp. No. | When Cut. | When Killed. | Number of Macronuclei, Number of Micronuclei. |
|---|---|---|---|
| 7 | 1 hr. 17 min. after division. | 3 hrs. 15 min. after cut. | 2 macronuclei, 2 micronuclei. |
| 22 | Before division | 1 hr. 10 min. " " | 1 macronucleus, 2 " |
| 25 | " " | 2 hrs. 25 " " " | 2 macronuclei, 2 " |
| 31 | " " | 2 " 30 " " " | 1 macronucleus, 1 micronucleus. |
| 32 | " " | 3 " 30 " " " | 1 " 1 " |
| 35 | " " | 11 " 40 " " " | 2 macronuclei, 2 micronuclei. |
| 47 | 6 hrs. 8 min. after division. | 14 " 47 " " " | 2 " 2 " |
| 51 | 6 hrs. 3 min. after division. | 13 " 3 " " " | 2 " 2 " |
| 57 | ——— | 1 hr. 35 " " " | 1 macronucleus, 1 micronucleus. (dividing) |
| 88 | Before division. | 14 hrs. 3 " " " | 4 macronuclei, 4 micronuclei. |
| 93 | " " | 41 " 30 " " " | 2 " 2 " |
| 95 | " " | 45 " 21 " " " | 2 " 2 " |

nearer the anterior end than normally. It is round in shape, measuring 5 micra in diameter. Its chromatin is homogeneous, deeply stained, showing no vesicles or granules. The posterior macronucleus is likewise situated nearer the anterior end than normally. It measures 7 x 5 micra and its chromatin is of the

TABLE IV.

ANTERIOR- AND POSTERIOR-END CUTS.

| Exp. No. | When Cut. | When Killed. | Number of Macronuclei, Number of Micronuclei. |
|---|---|---|---|
| 99 | Before division. | 43 hrs. 47 min. after cut. | 2 macronuclei, 2 micronuclei. |

same character as that of the anterior macronucleus. There is one micronucleus adjacent to each macronucleus and seven other micronuclei scattered irregularly throughout the anterior portion of the cell. They are all of about the same size, 2 micra in diameter, deeply stained and homogeneous. The cytoplasm

is finely granular. The changes here appear more like those of a degenerative than those of a physiological process and may have been present at the time the animal was operated upon.

In the twenty other experiments there were two macronuclei

TABLE V.

LONGITUDINAL CUTS.

| Exp. No. | When Cut. | When Killed. | Number of Macronuclei, Number of Micronuclei. |
|---|---|---|---|
| | | | (dividing) |
| 86 | Before division. | 20 hrs. 20 min. after cut. | 4 macronuclei, 4 micronuclei. |
| 110 | " " | 25 hrs. 50 min. after cut. | 2 macronuclei, 2 micronuclei. |

and two micronuclei, the number normally and most constantly found in this strain of animals.

In Table VI. are recorded the observations made on twelve animals, descendants of merozoa and selected from various generations from the second to the thirty-fifth. In all of these animals there were two macronuclei and two micronuclei.

TABLE VI.

| Exp. No. | Cut. | Generation after Cutting. | Number of Macronuclei, Number of Micronuclei. |
|---|---|---|---|
| 16 | Anterior. | 35th | 2 macronuclei, 2 micronuclei. |
| 21 | Posterior. | 30th | 2 " 2 " |
| 25 | Mid-body. | 24th | 2 " 2 " |
| 37 | Anterior. | 10th | 2 " 2 " |
| 53 | " | 2d | 2 " 2 " |
| 61 | Mid-body. | 4th | 2 " 2 " |
| 64 | Posterior. | 2d | 2 " 2 " |
| 64 | " | 3d | 2 " 2 " |
| 70 | " | 2d | 2 " 2 " |
| 70 | " | 3d | 2 " 2 " |
| 85 | Anterior. | 2d | 2 " 2 " |
| 106 | Posterior. | 2d | 2 " 2 " |

Thus, the evidence furnished by these experiments is that merotomy does not produce any change in the normal number of macronuclei and micronuclei, either in the merozoön or in its descendants as far as the thirty-fifth generation. The occurrence of cells with more micronuclei than macronuclei are more readily explained as having been mechanically produced by the operation itself or as the manifestation of an abnormal cell process that may have existed before merotomy was performed, as such cells are found in normal laboratory cultures of this animal.

## The Occurrence of Animals Containing but One Macronucleus.

On four occasions animals from the stock cultures and from the cultures of the control strains when killed and stained were found to possess but one macronucleus and sometimes one and sometimes two micronuclei. These animals were all large slow swimmers or crawlers, and were selected because experience had taught that these were the animals that showed early stages of division, which were being sought at that time.

Among the descendants of merozoön c, experiment 24, a uninucleate animal with two micronuclei was found. The original merozoön was from an animal cut during division. From this it was thought that perhaps injury to the cell during division might be the cause of this occurrence, but out of a large number of animals out of this strain and other strains derived from animals cut during division no other animals with a single macronucleus were found.

In experiment 76 a large slow crawler, evidently near division, was cut anteriorly at 12:25 P.M., August 9. The anterior merozoön disintegrated immediately, the posterior merozoön increased its activity. It was isolated into a clean glass capsule with five drops of tap water and one drop of hay infusion. On August 10, 10:15 A.M., there was found in the capsule three animals, two small and of the same size and a single large animal. The interpretation made of this was that the merozoön had regenerated, divided once into two individuals and that one of the latter had again divided while the other was now approaching division. On killing and staining all three of these animals it was found that each of the small animals had two macronuclei and two micronuclei, but the large animal had only one macronucleus and two micronuclei. The macronucleus appeared about to divide.

## Discussion.

Since uninucleate forms occur both among the stock cultures, the control strains and among the merozoa, a conservative inference must be that they are normal variations of this animal, possibly brought about by its being adapted to laboratory media. The relation between the two may be somewhat analogous to

that which Calkins[2] has found to exist between the so-called *Paramecium caudatum* and *Paramecium aurelia*, except that there it is a variation in the number of micronuclei; here, a variation in the number of macronuclei. It appears possible that by proper selection a strain of Pleurotricha may be obtained in which all of the animals, for a period, may show only one macronucleus.

From the experiments it appears fairly conclusive that merotomy generally has no effect on the normal number of micronuclei. Animals with less macronuclei than the normal may be found both in laboratory cultures of this animal and among the descendants of merozoa. Animals with more than the normal number of micronuclei may occur in laboratory cultures. The single instance in which there was found among the merozoa an animal with two abnormal macronuclei and several micronuclei is undoubtedly that of a degenerative or a necrobiotic cell. This is somewhat suggestive that multiple micronuclei may in general be one of the manifestations of a degenerative or a necrobiotic process in the cell.

It is to be noted that these observations are not in accord with Lewin's findings for *Stylonychia*. It appears from his paper that he had some difficulty in getting his animals to survive in the media used and another possible explanation for his observations is that they were more the results of necrobiosis and degeneration in the culture strains than the effect of merotomy.

## Conclusions.

1. Merotomy has no effect other than the effect that may be mechanically produced by the operation itself on the number of micronuclei of *Pleurotricha*.

2. Animals with more than the normal number of micronuclei and less than the normal number of macronuclei may occur in laboratory strains of this animal as well as among merozoa.

## Acknowledgments.

I am indebted to Dr. G. N. Calkins for suggesting this study to me and for assistance, also to Dr. George A. Baitsell for the

cultures of *Pleurotricha* with which the work was performed. To each of these men I desire to express my sincere appreciation.

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# THE REACTIONS OF CRAYFISHES TO GRADIENTS OF DISSOLVED CARBON DIOXIDE AND ACETIC AND HYDROCHLORIC ACIDS.[1]

EDWIN B. POWERS.

TABLE OF CONTENTS.



## I. Introduction.

The following experiments were undertaken to determine the reactions of crayfishes to gradients of acids, and, if possible, to determine the relation of the distribution of carbon dioxide contained in water to the natural distribution of the crayfishes. It was also hoped that something might be added to the present knowledge of the physiology of rapid modification of animals in

[1] From the Hull Zoological Laboratory, University of Chicago.

gradients. The experiments were conducted between January 13 and April 27, and between June 16 and July 31, 1913. The first set will be designated as low-temperature experiments and the second set as high-temperature experiments.

## II. Material and Methods.

### 1. *Apparatus and Method of Experimentation.*

In the study of reactions of the crayfishes in gradients of carbon dioxide and acetic and hydrochloric acids, the method and apparatus were devised by Shelford and Allee ('13) for the study of reactions of fishes to gases or solids in solution. The apparatus, a full description of which is given in Shelford and Allee ('13) "The Reactions of Fishes to Gradients of Dissolved Atmospheric Gases" (pp. 225–229), consists of two tanks each 120 cm. long by 20.5 cm. wide by 14 cm. deep with outlets at the center of both sides, near the top. These outlets are guarded by a screen-bottomed tube which extends across the tank. The tanks are placed side by side in an aquarium, beneath a hood under identical and symmetrically surrounding conditions. Tap water was introduced at both ends through perforated tees behind screens. In the experiments with carbon dioxide, the flows were 600 c.c. per minute. At one end carbon dioxide was introduced into the inlet so that a gradient was produced lengthwise of the tank between the tap water and the water high in carbon dioxide. This was shown by titrations made of samples taken from different portions of the tank. In the acetic and hydrochloric acid experiments the conditions were the same, except for the flow at the tap water end and corresponding end of the control, which was 1,200 c.c. per minute, thus producing a sharper gradient. This flow was also used in all high temperature carbon dioxide experiments. The acetic and hydrochloric acids were introduced by means of a separate tee.

After everything was made ready for the keeping of records, the crayfishes were dropped into the center of the tank and were observed through a slit in the hood, and the back and forth movements recorded in the form of a graph on paper especially prepared for this purpose. Records were also kept of specific reactions observed in any individual. These records were made

between the spaces set aside for the graphs. The previous history of the animals and the gradient used with a description of conditions of experimentation were entered at the top of the page. After the crayfishes were dropped into the middle of the tank they were disturbed as little as possible. At the end of each experiment a titration of samples of water taken from the two ends and center was made with sodium carbonate and recorded.

### 2. *Stock.*

The crayfishes were of the species *Cambarus propinquus* Gir., *C. virilis* Hag., *C. diogenes* Gir. and *C. immunis* Hag. They were all of medium size with the exception of a few specimens of *C. virilis* and *C. diogenes* which were above medium size. *C. propinquus* and *C. virilis* were all taken at New Lenox, Ill., from the pools just above and just below the rapids and from among the rocks of the rapids of Hickory Creek. The first stock was obtained December 25, 1912, and was kept in the laboratory in a large pan with the bottom covered with sand, gravel and some vegetation. The water was changed six times per week. When brought in a few died during the first three to five days, due possibly to the sudden change of temperature, but after this there was little mortality. Stock obtained November 15, 1912, and January 25, 1913, which were kept in an aquarium, suffered the same experience when transferred to the pan. Stock obtained March 22 and 29 and June 21, 1913, was kept in an aquarium in which was a strong flow of water to lower the temperature to more nearly that of experimentation. These suffered no great mortality. *C. immunis* was obtained from sloughs, mostly from the bottom, and a few from burrows, and *C. diogenes* from burrows only, along the banks of the same sloughs near Clark Junction, Ind., June 20 and 24 and July 9 and 26. These were all kept in large glass jars into which fresh water was flowing. There was no great mortality experienced by these stocks.

### 3. *Habitat.*

*C. propinquus* is essentially a quiet water stream form. It inhabits the pools with more or less muddy bottom (Williams, '01). They hide under rocks or rest quietly on the bottom.

Sometimes they lie concealed in short burrows along the banks (Harris, '03). *C. virilis* is found to be absent from the muddier and shallower portions of the streams but is sometimes taken in ponds with *C. immunis* (Harris, '01), but is more often found in running streams, among the rocks of the more rapid portions (Harris, '03). They do not burrow except when the ponds begin to dry or winter approaches. Garman ('89) took this species from wells, Wilson's cave and streams. *C. diogenes* is a burrowing species and often makes mud chimneys, often burrowing in damp ground some distances from the open water, which it seldom enters except during the breeding season (Pearse, '10). *C. immunis* is a mud-loving species and is found mostly in small pools, though it sometimes occurs in brooks and rivers (Pearse, *l. c.*) (Harris, '03).

4. *Senses.*

The fact that crayfishes are sensitive to chemicals has been shown by several authors. Putnam ('75) noted that *C. pellucidus*, the blind crayfish, will hunt food when it is thrown into the water. Holmes and Homuth ('10) found that the whole body is more or less sensitive to olfactory stimuli, and that the antennæ, mouth parts and tips of the chelipeds are sensitive in the order named. Nagel ('94) observed the chemical sense in *Astacus*. Wright ('84), by a study of the antennules of *C. propinquus*, found that five of the eighteen segments, *i. e.*, eleven to fifteen inclusive, bear eight of the so-called olfactory organs and the distal nine fewer. Bell ('06) found that the crayfishes with which he worked reacted positively to meat juice and negatively to lavender water, acids and salts, and concluded that they are sensitive over the entire body but more in the anterior appendages than in other parts. Chidester ('12) found when meat was thrown into the water that crayfishes would approach and seize fresh meat quicker than they would meat dried in the air.

## III. The Sensing of Carbon Dioxide and Acetic and Hydrochloric Acids.

The crayfishes sensed the carbon dioxide and acetic and hydrochloric acids when passing into the high acid concentration end of the experiment tank. This was indicated by certain specific

reactions which were made as the crayfishes entered the higher concentration. Such reactions were waving the antennæ, moving the appendages, backing and walking with the legs extended. The waving and moving of the appendages were especially noted in the reactions of *propinquus*, *virilis* and *diogenes*, in the carbon dioxide experiments in which the concentrations of carbon dioxide in the two ends were 40 to 60 and 80 to 100 c.c. per liter respectively. The animal would also crouch down in the corner of the tank. This tendency to wave the antennæ and move the appendages was present in *virilis* in the acetic acid also. The backing reaction was not so common in the carbon dioxide experiments but was marked in the hydrochloric and acetic acid experiments.

## IV. The Effect of Carbon Dioxide and Acetic and Hydrochloric Acids.

### 1. *The Effect on Reflex of the Crayfishes.*

The crayfishes not only detected the presence of the acids but were intoxicated or anæsthetized by them in the low temperature experiments, possibly due to the less regular movements of the animal. This is shown in graphs (Chart I.) by the longer periods of time required to cross the tank in the experiment than that required in the control. The effect was greatest in the experiments with high concentrations. The first effect of the carbon dioxide was to interfere with the correlation of movements and to cause the animal to carry the body high with legs extended. Progressively locomotion became slower and slower until it ceased, but the appendages were still moved one after the other. The crayfish would finally fall upon its back and continue to move its appendages for a short time, after which it would remain motionless as if dead. It would recover rapidly if placed in fresh water and after a short time move about normally. The acetic acid produced this same effect upon *virilis* but *propinquus*, *diogenes* and *immunis* were either not intoxicated by the acetic and hydrochloric acids or acted rapidly enough to get out of the high concentration before being greatly affected, (see Charts I. and II.). The movements of *virilis* were always more or less irregular. One of the individuals in experiment 21

was completely anæsthetized but recovered in eight minutes when placed in fresh water. At first it remained motionless in the fresh water but later revived and seemingly became normal.

### 2. *Anæsthesia and Death.*

An experiment was performed to determine the relative resistance of the four species of crayfishes to high concentration of carbon dioxide. Though the experiment was terminated before the crayfishes had all been killed, through an accident to the apparatus, it had been carried sufficiently far to determine the susceptibility of the four species to the carbon dioxide solution. The apparatus consisted of large glass bottles (Wells, '13) through which water containing from 6.92 to 7 c.c. of oxygen per liter and varying amounts of carbon was flowing. The amount of the gases were determined by titrations of samples of water collected from the over-flow. The temperature was 21.5° to 23° C.

It was found that in all cases the smaller individuals of a species died first. This was probably due to the greater proportion of surface to mass in the smaller specimens than in the larger ones, rather than a difference of susceptibility of the smaller specimens to the carbon dioxide solution. These are the same general results obtained by Wells (Wells, *l. c.*) with fishes.

All remained active in 50 c.c. carbon dioxide per liter. *Virilis* was anæsthetized by solutions of 120 to 145 c.c. per liter. *Propinquus* was not overcome but showed that they were effected, while *diogenes* and *immunis* showed the effect to a less extent. *Propinquus* was not anæsthetized as early as *virilis* but the time of death approached that of *virilis*, the small specimens of *propinquus* having died before the large *virilis*, but a medium sized *propinquus* survived all the specimens of *virilis*. *Diogenes* and *immunis* were much less susceptible to the carbon dioxide than either *propinquus* or *virilis*. All specimens of both *propinquus* and *virilis* died before the medium-sized specimen of *diogenes*. The specimens of *diogenes* and *immunis* died in the following order: One medium-sized *diogenes*, one *immunis*, one *diogenes*, one *immunis*, two *diogenes* and two of *immunis*. There were one *immunis* and one large *diogenes* alive when the experi-

ment terminated. All the specimens of *immunis* were smaller in size than the smallest specimen of *diogenes*. From this data it seems the four species are susceptible to high concentrations of carbon dioxide in the following order: *virilis*, *propinquus*, *diogenes* and *immunis*.

## V. The Reaction and Modification in Gradients.

The reaction of crayfishes in gradients is shown in Tables I. and II. Table I. for carbon dioxide, II. for acetic and hydrochloric acids, are arranged in order of sharpness of the gradient, *i. e.*, the difference between the concentrations of acid at the two ends. The four species are grouped separately in each table. Reaction is shown by the time preference for one end or the other, by turnings and by crossings of the center. There is also tabulated the modification of behavior by turnings accompanied by backing. The backing reaction is not indicated in the ratings (Table III.), which is the numerical expression of avoidance of ends when turnings and time spent in the halves of the tank are considered. The ratio of the increased concentration of the acid of the high end over that of the low end, or Weber's ratio, is tabulated for comparison with the difference of concentration of the acid at the two ends.

The crayfishes, when passing into the high concentration end of the tank, gave certain definite avoiding reactions, when not too much affected by the presence of the acid. Of these the reactions recordable in graphs are (1) turning upon encountering gradient, either the first time or after one or more invasions and (2) reactions which cannot be recorded graphically are turning accompanied by backing and crawling on the screen, out of the water, or attempting to crawl on the sides of the tank. Recordable reactions were in the most cases rhythmic and represented a rapid modification of behavior. Reactions of the first class are grouped in one column of the table, but where backing accompanied turning separate mention is made of this fact. Of the reactions that could not be graphically recorded, that of crawling on the screen is least definite as an avoiding reaction since it was noted in the controls also, though less than in the experiments. This reaction is probably due to thigmotactic

## Table I.

Showing reaction of crayfishes in a gradient of carbon dioxide. In all experiments and controls three animals were tested simultaneously except in the 20-minute experiments and controls in which case each of the three individuals were tested separately, 20 minutes each. H indicates high carbon dioxide and L low carbon dioxide. Corresponding ends of control tank are designated by the same letters. Typical experiments, only, are given in table. Totals and per cent. are totals for all experiments and controls of each species of crayfishes in each gradient. The experiments of each species are grouped and arranged in order of sharpness of gradient between the two ends of tank. For gradient see Table III.

| Experiment No. | *Cambarus* Species. | Carbon Dioxide in c.c. per L. | | Per Cent. of Time Spent in Halves of Experimental and Control Tanks. | | | | Number of Times Crossed Center. | | Number of Times Turned in Gradient from High and Low Ends. | | | | Number of Turnings Accompanied by Backings. | | | | Temperature, Centigrade. | Time of Experiment. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Expt. | | Cont. | | | | Expt. | | Cont. | | Expt. | | Cont. | | | |
| | | L | H | H | L | H | L | Expt. | Cont. | H | L | H | L | H | L | H | L | | |
| 2 | *Propinquus*.. | 3.5 | 20. | 27.9 | 72.1 | 51. | 49. | 23 | 40 | 17 | 4 | 3 | 2 | 5 | 0 | 0 | 1 | 6. | 60 |
| 26 | " | 3.6 | 23. | 21.3 | 78.7 | 37.1 | 62.9 | 48 | 36 | 31 | 7 | 7 | 12 | 0 | 0 | 0 | 0 | 17.5 | 40 |
| 5 | " | 47.4 | 94. | 5.6 | 94.4 | 70.6 | 29.4 | 7 | 41 | 12 | 1 | 1 | 5 | 4 | 0 | 0 | 0 | 3.75 | 60 |
| | Totals..... | | | | | | | 157 | 237 | 124 | 57 | 32 | 40 | 20 | 3 | 3 | 1 | | |
| | Per cent..... | | | | | | | | | 71 | 29 | 44.5 | 55.5 | | | | | | |
| 24 | *Virilis*..... | 1.5 | 9 5 | 41.7 | 58.3 | | | 39 | | 30 | 23 | | | 2 | 0 | | | 19. | 40 |
| 27 | " ..... | 2.9 | 46.5 | 11.1 | 88.9 | 24.1 | 75.9 | 40 | 18 | 40 | 21 | 14 | 20 | 1 | 0 | 0 | 0 | 17.5 | 40 |
| | Totals..... | | | | | | | 108 | 85 | 78 | 54 | 27 | 24 | 4 | 0 | 0 | 0 | | |
| | Per cent..... | | | | | | | | | 59 | 41 | 53 | 47 | | | | | | |
| 44 | *Diogenes*..... | 2.3 | 18.9 | 33.3 | 66.7 | 35.8 | 64.2 | 18 | 21 | 7 | 5 | 5 | 3 | 0 | 0 | 0 | 0 | 20.5 | 20 |
| 28 | " .... | 56.5 | 179.4 | 26. | 74. | 58.8 | 41.2 | 20 | 32 | 18 | 11 | 4 | 3 | 8 | 2 | 0 | 1 | 17.5 | 20 |
| | Totals..... | | | | | | | 58 | 85 | 69 | 45 | 39 | 29 | 15 | 6 | 0 | 1 | | |
| | Per cent..... | | | | | | | | | 60 | 40 | 57.3 | 42.7 | | | | | | |
| 45 | *Immunis*. .... | 2.3 | 9.4 | 33.7 | 66.3 | 48.3 | 51.7 | 52 | 38 | 13 | 5 | 7 | 8 | 0 | 0 | 0 | 0 | 21. | 20 |
| 46 | " .... | 2.7 | 24.5 | 48.5 | 51.5 | 54.6 | 46.4 | 37 | 37 | 13 | 11 | 1 | 2 | 1 | 2 | 0 | 0 | 20.5 | 20 |
| | Totals..... | | | | | | | 139 | 65 | 49 | 33 | 8 | 10 | 2 | 3 | 0 | 0 | | |
| | Per cent..... | | | | | | | | | 59.8 | 40.2 | 44.5 | 55.5 | | | | | | |

response as well as an avoidance of the acids. The tendencies to crawl out of the water and upon the side of the tank are more clearly acid avoiding reactions. In the controls there were no attempts to crawl on the sides of the tank and few animals showed an inclination to crawl out of the water.

### 1. *Carbon Dioxide Gradient.*

(*a*) *C. propinquus.*—The crayfishes, upon invading the high carbon dioxide end, showed a tendency to turn before reaching the screen, or they would back a short distance and then turn and pass to the lower carbon dioxide end. Of all the individuals tried 55 per cent. turned back on the first encounter of the gradient and 14.8 per cent. of these turnings were accompanied by backings (Table I.). There was a greater number of turnings from the high concentration end than from the low, there being a total of 124 from the high end to 51 from the low. The fact that turning from the high end is an avoiding reaction is emphasized by 16 per cent. of the turnings being accompanied by backing. There were fewer crossings of the center in the experiments than in the controls, in the latter there being a tendency to travel the entire length of the tank. In some cases the reduction of crossings of the center represents the extent of anæsthetization of the animals.

In the experiments with low concentrations (low 3.5 and high 20 c.c. of carbon dioxide per liter) and low temperature the turnings were rhythmical (Chart I., Expt. 2). Long invasions of the high concentration end were followed by shorter invasions and periods of rest at the low concentration end. These were then followed by very short invasions and very long periods of almost complete rest in the low concentration end. Later there would be a second similar period of invasion and rest. Three such periods are shown in the graph. Thus there is a period of increased sensitiveness or a period of increased reaction to the same incoming sensation, after invasion of the high concentration end. In either case there is a rapid modification of behavior.

With higher concentrations (24–47 c.c. per L., low end and 58–94 c.c. per L. high end), if the crayfishes were not too greatly overcome, there was greater rapidity of reaction and a more

## TABLE II.

Showing reaction of crayfishes in gradients of acetic and hydrochloric acids. For further explanation see Table I.

| Experiment No. | *Cambarus* Species. | Acetic Acid in g. per L. | | Per Cent. of Time Spent in Halves of Experimental and Control Tanks. | | | | Number of Times Crossed Center. | | Number of Times Turned in Gradient from High and Low Ends. | | | | Number of Turnings Accompanied by Backings. | | | | Temperature, Centigrade. | Time of Experiment. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Expt. | | Cont. | | | | Expt. | | Cont. | | Expt. | | Cont. | | | |
| | | L | H | H | L | H | L | Expt. | Cont. | H | L | H | L | H | L | H | L | | |
| 14 | *Propinquus*.. | .24 | 1.39 | 3.5 | 96.5 | 54.9 | 45.1 | 14 | 22 | 15 | 3 | 11 | 13 | 7 | 0 | 0 | 0 | 4.25 | 60 |
| 13 | " .. | 2.41 | 3.66 | | 100. | 61.3 | 38.7 | 0 | 16 | 4 | 0 | 6 | 6 | 2 | 0 | 0 | 0 | 4.25 | 60 |
| | Totals..... | | | | | | | 45 | 93 | 81 | 15 | 33 | 34 | 33 | 0 | 1 | 0 | | |
| | Per cent..... | | | | | | | | | 84.5 | 15.5 | 49 | 51 | | | | | | |
| 21 | *Virilis*..... | 1.21 | 1.67 | 41. | 59. | 45.3 | 54.7 | 11 | 13 | 17 | 7 | 11 | 5 | 9 | 1 | 1 | 0 | 6.25 | 40 |
| 30 | " ..... | .266 | 3.926 | 3.2 | 96.8 | 28.2 | 71.8 | 11 | 15 | 22 | 6 | 6 | 7 | 7 | 0 | 0 | 2 | 16.5 | 40 |
| 35 | " ..... | 2.88 | 28.99 | 23.2 | 76.8 | 53.8 | 46.2 | 11 | 24 | 13 | 2 | 6 | 1 | 11 | 1 | 2 | 0 | 17. | 20 |
| | Totals..... | | | | | | | 101 | 139 | 113 | 46 | 51 | 40 | 52 | 7 | 3 | 5 | | |
| | Per cent..... | | | | | | | | | 71 | 29 | 36 | 44 | | | | | | |
| 31 | *Diogenes*..... | .086 | .312 | 10. | 90. | 63.9 | 36.1 | 21 | 27 | 15 | 2 | 11 | 5 | 3 | 0 | 1 | 1 | 16.5 | 20 |
| 33 | " .... | .014 | .354 | 41.1 | 58.9 | 92. | 8. | 26 | 10 | 19 | 10 | 18 | 2 | 5 | 3 | 4 | 0 | 16.5 | 20 |
| | Totals..... | | | | | | | 93 | 117 | 51 | 29 | 43 | 24 | 14 | 10 | 5 | 2 | | |
| | Per cent..... | | | | | | | | | 64 | 36 | 64 | 36 | | | | | | |
| 37 | *Immunis*.... | .078 | .429 | 29.4 | 70.6 | 53.8 | 46.2 | 21 | 45 | 24 | 8 | 11 | 9 | 7 | 2 | 0 | 0 | 18. | 20 |
| 34 | " .... | .429 | 1.437 | 16.7 | 83.3 | 54.1 | 45.9 | 6 | 3 | 5 | 5 | 1 | 3 | 2 | 0 | 0 | 0 | 16.5 | 20 |
| | Totals..... | | | | | | | 33 | 52 | 34 | 15 | 14 | 16 | 10 | 3 | 0 | 1 | | |
| | Per cent..... | | | | | | | | | 67.4 | 32.6 | 46.7 | 53.3 | | | | | | |

TABLE II.—*Continued.*

| Experiment No. | *Cambarus* Species. | Hydrochloric Acid in g. per L. | | Per Cent. of Time Spent in Halves of Experimental and Control Tanks. | | | | Number of Times Crossed Center. | | Number of Times Turned in Gradient from High and Low Ends. | | | | Number of Turnings Accompanied by Backings. | | | | Temperature, Centigrade. | Time of Experiment. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Expt. | | Cont. | | | | Expt. | | Cont. | | Expt. | | Cont. | | | |
| | | L | H | H | L | H | L | Expt. | Cont. | H | L | H | L | H | L | H | L | | |
| 39 | *Propinquus*.. | .0061 | .0157 | 9.4 | 90.4 | 60.1 | 39.9 | 29 | 30 | 27 | 6 | 1 | 2 | 4 | 0 | 0 | 2 | 20.5 | 20 |
| 47 | " .. | .0275 | .0932 | 4.7 | 95.3 | 49.6 | 50.4 | 28 | 63 | 39 | 2 | 0 | 1 | 3 | 0 | 0 | 0 | 21. | 20 |
| | Totals..... | | | | | | | 57 | 93 | 66 | 8 | 1 | 3 | 7 | 0 | 0 | 3 | | |
| | Per cent..... | | | | | | | | | 89.2 | 10.8 | 25 | 75 | | | | | | |
| 41 | *Virilis*..... | .0075 | .067 | 12.4 | 87.6 | 49.3 | 50.7 | 11 | 32 | 24 | 8 | 2 | 6 | 14 | 2 | 0 | 2 | 20.5 | 20 |
| | Per cent..... | | | | | | | | | 75 | 25 | 25 | 75 | | | | | | |
| 42 | *Diogenes*..... | .0023 | .0078 | 30.5 | 69.5 | | | 39 | | 7 | 5 | | | 2 | 0 | | | 21. | 20 |
| 43 | " .... | .0053 | .0702 | 13.8 | 86.2 | 39.3 | 62.7 | 18 | 49 | 14 | 6 | 11 | 13 | 2 | 0 | 0 | 3 | 21. | 20 |
| | Totals..... | | | | | | | 57 | 49 | 21 | 11 | 11 | 13 | 4 | 0 | 0 | 3 | | |
| | Per cent..... | | | | | | | | | 66.7 | 33.3 | | | | | | | | |
| 40 | *Communis*... | .0014 | .0304 | 31.5 | 68.5 | 49.3 | 50.7 | 23 | 42 | 18 | 7 | 10 | 8 | 2 | 1 | 0 | 1 | 20.5 | 20 |
| | Per cent..... | | | | | | | | | 72 | 28 | 44.5 | 55.5 | | | | | | |

## TABLE III.

Showing the vigor of reaction or rating of the crayfishes in gradients of carbon dioxide and acetic and hydrochloric acids. The rating is obtained by subtracting the percentages given for the time preference for the two ends and subtracting the percentage of turnings from the two ends and adding the two remainders (which are considered of different signs since turnings are from opposite end to end preferred) and dividing by two. The table also shows difference in concentration between the two ends and the ratio of increase of gradient at high end over low end or Weber's ratio.

| Experiment No. | *Cambarus* Species. | Grams per L. of Acid or c.c. per L. $CO_2$. | | Difference in g. or c.c. per L. between Two Ends of Tank. | Weber's Ratio. | Rating. | | |
|---|---|---|---|---|---|---|---|---|
| | | L | H | | | Expt. | Cont. | |
| 22 | *Propinquus* | 1.5 | 11.8 | 10.3 | 6.8 | 7. | | |
| 2 | " | 3.5 | 20. | 16.5 | 4.7 | 53. | 9. | |
| 26 | " | 3.6 | 23. | 19.4 | 5.4 | 60. | 26. | |
| 8 | " | 120. | 149. | 29. | .24 | 32. | 59. | |
| 3 | " | 24. | 58. | 34. | 1.4 | 40. | 23. | Carbon dioxide |
| 5 | " | 47.4 | 94. | 46.6 | .98 | 86.0 | 53. | gradient. |
| 4 | " | 43. | 90. | 47. | 1.09 | +5.5 | 20. | |
| 6 | " | 32. | 82. | 50. | 1.5 | 32. | 59. | |
| 29 | " | 37.1 | 154.7 | 117.6 | 4.3 | 42. | 32. | |
| 24 | *Virilis*.... | 1.5 | 9.5 | 8. | 6.3 | 24. | | Ratio of increase |
| 10 | " | 18. | 54. | 36. | 2. | +43. | 46. | of gradient at |
| 11 | " | 62.5 | 101. | 38.5 | .26 | 2. | 54. | high end over |
| 27 | " | 2.9 | 46.5 | 43.6 | 15. | 54. | 17. | low end or |
| 9 | " | 86. | 172. | 86. | 1. | 38. | 3. | Weber's ratio. |
| 44 | *Diogenes*... | 2.3 | 18.9 | 16.6 | 7.6 | 24. | 27. | |
| 25 | " | 3.3 | 35.9 | 32.6 | 9.9 | 13. | 1.5 | |
| 28 | " | 56.5 | 179.4 | 122.9 | 2.1 | 36. | 1. | |
| 45 | *Immunis*.. | 2.3 | 9.4 | 7.1 | 3. | 38. | 2. | |
| 23 | " | 1.3 | 9.6 | 8.3 | 6.4 | + 4. | | |
| 46 | " | 2.7 | 24.5 | 21.8 | 7.7 | 4. | 21. | |
| 17 | *Propinquus* | .045 | .183 | .138 | 3.2 | 79. | 25. | |
| 16 | " | .045 | .3 | .255 | 5.7 | 83. | 9. | |
| 15 | " | .24 | .69 | .45 | 1.9 | 65. | 7. | |
| 14 | " | .24 | 1.39 | 1.15 | 5.2 | 79. | 8. | |
| 13 | " | 2.41 | 3.66 | 1.25 | .48 | 100. | 11. | |
| 18 | *Virilis*.... | .018 | .114 | .096 | 5.1 | 53. | 11.6 | |
| 19 | " | .018 | .22 | .202 | 11.3 | 52. | 25. | Acetic acid gradi- |
| 21 | " | 1.21 | 1.67 | .46 | .3 | 39. | 8.6 | ent. |
| 20 | " | .122 | .8 | .67 | 5.5 | 15. | 10. | |
| 30 | " | .266 | 3.926 | 3.66 | 13.7 | 75. | 21. | |
| 35 | " | 2.88 | 28.99 | 26.11 | 9. | 63. | 16. | |
| 31 | *Diogenes*... | .086 | .312 | .226 | 18.5 | 78. | 1.4 | |
| 33 | " | .014 | .354 | .3406 | 23.8 | 24. | 92. | |
| 38 | " | 1.027 | 2.938 | 1.911 | 18. | .6 | 2.2 | |
| 36 | " | 4.97 | 23.33 | 18.36 | 3.6 | 8. | 4.7 | |
| 32 | *Immunis*.. | .0109 | .1105 | .0996 | 9.1 | 38. | 20. | |
| 37 | " | .078 | .429 | .351 | 4.5 | 45. | 1.2 | |
| 34 | " | .429 | 1.437 | 1.008 | 2.3 | 33. | 29. | |
| 39 | *Propinquus* | .0061 | .0157 | .0096 | 1.6 | 72. | 26. | |
| 47 | " | .0275 | .0932 | .0657 | 2.3 | 90. | 45. | |
| 41 | *Virilis*.... | .0075 | .067 | .0595 | 7.9 | 63. | 12. | |
| 42 | *Diogenes*... | .0023 | .0078 | .0055 | 2.4 | 28. | | Hydrochloric acid |
| 43 | " | .0053 | .0702 | .0649 | 12.2 | 56. | 7. | gradient. |
| 40 | *Immunis*.. | .0014 | .0304 | .029 | 20.2 | 40. | 6. | |

rapid modification of behavior (Chart I., Expt. 5). Periodicity is still present but is marked by shorter invasions of the high end. This periodicity is better shown by individuals than by groups. The graph of experiment 5, Chart I., shows that there was a complete cessation of invasions of the high end after 48 minutes and the crayfishes were still resting in the low end after 80 minutes. Numerically expressed, 70 per cent. of all individuals tried showed modification.

(*b*) *C. virilis.*—*Virilis* oriented less definitely to the carbon dioxide gradient than *propinquus*. (Compare ratings, Table III.) The lack of orientation is shown by the percentage of turnings from the two ends and the time preference for one end or the other. This staggering is possibly due to the somewhat more concentrated solutions used, but may be explained as has been suggested by Shelford and Allee ('13) for swift water fishes. That is swift water fishes which encounter very little carbon dioxide may react less definitely to it than fishes which live more often in the presence of carbon dioxide. *Virilis* showed a tendency to crawl out of the water.

(*c*) *C. diogenes.*—*Diogenes* reacted less intensely to both the lower and higher concentrations of carbon dioxide in gradients than either *propinquus* or *virilis*. In experiment 44 with 18.9 c.c. carbon dioxide per liter at the high end there was no backing accompanying the turnings, while in experiment 28, with 179.4 c.c. carbon dioxide per liter at the high end, there were 44 per cent. of the turnings accompanied by backing and one of the turnings on first encounter was accompanied by backing. *Diogenes* also showed a marked tendency in the high concentration carbon dioxide gradient to move forward and then stop after which it would move forward again.

(*d*) *C. immunis.*—All experiments with *immunis* were with concentrations not running above 25 c.c. carbon dioxide per liter at the high concentration end. The avoiding reactions with the exception of experiment 45 were very low. There was a slight positive reaction in experiment 23 as is shown in Table III. There were two turnings from the high concentration end accompanied by backing in experiments 23 and 46 while at the same time there were 3 from the low end.

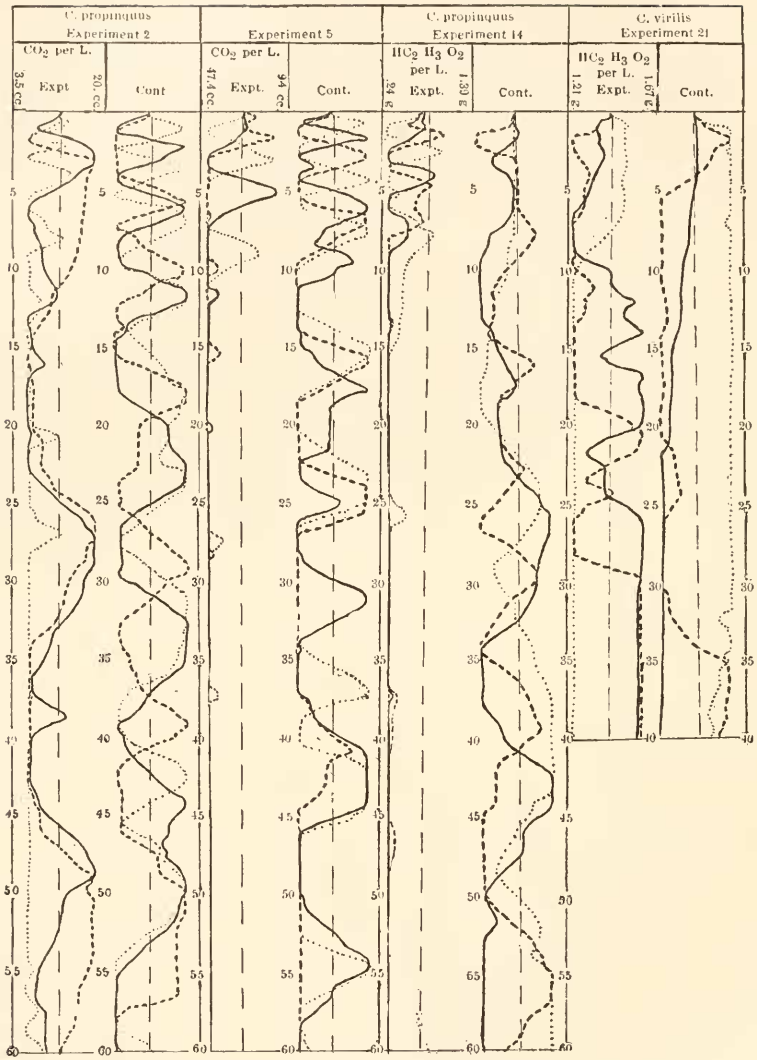


CHART I. Showing reaction of the crayfishes of gradients to carbon dioxide and acetic acid. The horizontal distance between the scales represents the length of the tank. The vertical scale represents time in minutes. Different tracings represent individual animals. Three animals were tested simultaneously.

### 2. *Acetic Acid Gradient.*

(*a*) *C. propinquus.*—The avoidance of the acetic acid in gradient was sharp and definite (see Chart I., Expt. 14). Orien-

tation was also definite. There seemed to be an acceleration of all the reactions shown in the carbon dioxide experiments. Out of fifteen trials fourteen (93.3 per cent.) showed turning at first encounter of the high concentration, and of the fourteen, four started toward the high end without first having invaded the low or even a portion of it; 50 per cent. of the turnings were accompanied by backing. Only two individuals reached the screen in the acetic acid end. Of these two one crossed after handling; the other occurred in the lowest concentration of acetic acid used. There was, after a certain period of time, a cessation of invasions of the high acid concentration end; this period varied inversely as the total concentration of the acid. Experiment 14 varies slightly from this rule. There was also periodicity of invasions of the high concentration end (see Chart I., Expt. 14) as described in the carbon dioxide experiments.

(*b*) *C. virilis.*—These experiments can be divided into three groups, a low concentration, a high and a very high, *i. e.*, experiments 18 and 19 with a concentration of .0185 g. of acetic acid per liter at the low end and .1145 to .228 g. per liter at the high end; experiments 20 and 21 with .122 to 1.21 and .8 to 1.67 g.; and experiment 35 with 2.88 and 28.99 g. per liter of acetic acid in the low and high ends respectively.

In the low concentration experiments at low temperature there was a more definite orientation and a greater time preference for the low end. This is shown by the ratings (see Table III.). There was also a more or less periodicity of invasions of the high end with a complete cessation of invasions in experiment 19 after 45 minutes.

In the high concentration experiments at low temperature there was less orientation except in turning at the first invasion of the high end. There was but one individual (Expt. 20) that showed periodicity of invasions of the high end. The lack of orientation is shown by graph Expt. 21, Chart I. There was an increased tendency to crawl on the sides of the tank and out of the water. Thus there was a falling off of orientation and a substitution of crawling out of the water.

In experiment 30, in which the temperature was high and specimens above medium size were used, there was better orienta-

tion with a greater intensity of avoiding reactions as is shown by the rating and per cent. of time spent in the halves of the pan. See Tables II. and III., and Chart II., graph 30. While in experiment 35 where the concentration was very high there was a falling off of the intensity of the avoiding reaction due to one specimen becoming more or less anæsthetized. See Table II. and Chart II., Expt. 35.

(*c*) *C. diogenes.*—In the acetic acid experiments as well as the carbon dioxide experiments the intensity of the avoiding reactions are rather low with the exception of experiment 31 which is rather high, the rating being 78. There was a stronger tendency to stop and then move forward and with longer periods of rest than was noted in the carbon dioxide experiments.

(*d*) *C. immunis.*—The avoiding reactions of *immunis* to acetic acid was definite in the time spent in the halves of the pan, by turnings and by turnings accompanied by backings. See Table II., Chart II., Expt. 37 and 34. The intensity of the reactions in the acetic acid as well as in the carbon dioxide was rather low as is shown by Tables I. and II.

### 3. *Hydrochloric Acid Gradient.*

After having completed the experiments with carbon dioxide and acetic acid it was thought advisable to test the crayfishes with some inorganic acid and thus determine the difference or similarity of the reactions of the crayfishes to the different acids and to better compare the reactions of the four species of crayfishes. Hydrochloric acid was selected for this purpose. Since the ion constant of hydrochloric acid in very weak concentrations is approximately one (Stiegletz, '11) very low, concentrations were used in all the experiments. By an inspection of the tables it is seen that the same intensity of avoiding reaction was obtained in the low concentrations of hydrochloric acid but of high ion concentration as was obtained by the higher concentrations of acetic acid and carbon dioxide of lower ion concentration. Not only was the intensity of the avoiding reactions high in proportion to the concentration, but the reaction of all four species was more definite as is indicated by the turnings from the ends and turnings accompanied by backings. See Table II. and Chart II. Expt. 47, Chart II. was extended over a period

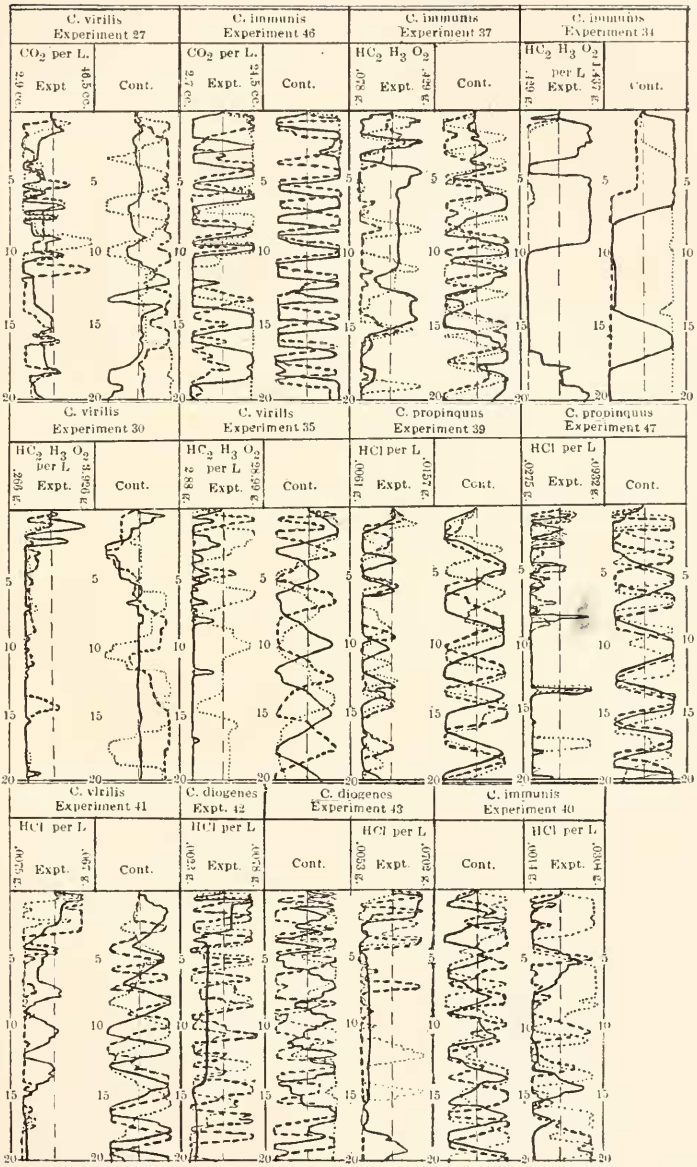


CHART II. Showing reaction of the crayfishes to gradients of carbon dioxide and acetic and hydrochloric acids. The horizontal distance represents the length of tank. The vertical distance represents time in minutes. Different tracings represent individual animals. In all experiments except experiments and controls 27 and 47 each animal was tested separately, 20 minutes each.

of forty minutes to determine the modification of behavior over a longer period of time. It was found that there was a similar rhythm of invasions of the high concentration end with a final coming to rest at the low acid concentration end.

4. *A Comparison of the Reactions of the Four Species of Crayfishes Tested.*

While there were noted specific differences in the reactions of the four species of crayfishes tested, all sensed the carbon dioxide and acetic and hydrochloric acids. Observations show that *propinquus* gives specific reactions and orients to a gradient of these substances, while *virilis* orients to a less degree; *immunis* to high concentrations, and *diogenes* to a still less degree to both high and low concentrations. Both *propinquus* and *virilis* which were tested at low temperatures were affected by carbon dioxide to the extent of intoxication, and *virilis* was affected more or less by the acetic acid. All species, so far as their avoiding reactions were noted, showed modifications of behavior and with *propinquus* there was a tendency to come to rest in the low end.

## VI. General Discussion.

In reviewing the data it is seen that in each set of experiments all the types of behavior are increased in intensity with increase in the concentration of acid used, and the question as to the relation between the cause of the different types of behavior is suggested.

In the first place there must be a gradient before there can be orientation. This is in accord with Weber's law which states: "The increase of the stimulus necessary to produce an increase of the sensation bears a constant ratio to the total stimulus,"[1] *i. e.*, there must be a definite ratio between the increased intensity of the stimulus and the original stimulus before there can be a sensation of an increased stimulus. If the crayfishes were reacting in accordance with this law, the rating, which is the numerical expression of the degree of the reaction when both turnings and time preference are considered, should be in proportion to Weber's ratio, see Table III. By turning to the experiments

[1] James, The Principles of Psychology.

with *propinquus* in carbon dioxide and comparing the ratios of the increased concentrations at the high acid concentration end with the concentration at the low end of the low temperature experiments, it will be seen that there is no definite relation between the two. This comparison may be objected to on the grounds that *propinquus* was intoxicated by the carbon dioxide, but by turning to the acetic acid experiment, where *propinquus* was not affected there is seen the same variation between the ratings and Weber's ratio. At the same time it will be seen that there is a more definite relation between the rating and the total concentration of the acetic acid. In general the lowest total concentrations of acids have the lowest ratings. Experiments 15 and 16 are exceptions but still the range is not wide as compared with the great variation of Weber's ratios.

Now turning back to the carbon dioxide experiments (Table III., Expts. 2, 26 and 5) the intensities of the reactions are in reverse proportion to Weber's ratios, but are in direct proportion to the concentrations of the carbon dixoide solution used in the experiments. In the hydrochloric acid experiments (see Chart II. and Table III., Expt. 39 and 47) the intensity of the reactions conform with Weber's ratio, but at the same time it conforms with the concentrations of the hydrochloric acid used. Thus it is seen that the total concentration of the acid determines the intensity of the reaction. In other words the intensity of the reaction varies directly as the hydrogen ion concentration. This view is supported by comparing the ratings of the carbon dioxide and acetic and hydrochloric acid experiments. It is seen by comparing the carbon dioxide and acetic and hydrochloric acid experiments (see Table III.) that the rating on an average of the hydrochloric acid experiments are highest, acetic acid next and carbon dioxide lowest. This is due not to the higher molecular concentration of the hydrochloric acid over that of the acetic acid, since the molecular concentrations of the acetic acid were higher than that of the hydrochloric acid (Tables I. and II.), but to the higher ionization of hydrochloric acid over that of acetic acid and acetic acid over that of carbon dioxide in solution, thus giving higher ion concentrations.[1] These same

[1] Stieglitz's table of the ionization constants of acids, 1911.

points are suggested by the comparison of the ratings of each of the other three species in the hydrochloric acid, acetic acid and carbon dioxide experiments. It is interesting to note that when turnings only are considered that in all four species in the carbon dioxide, acetic acid and hydrochloric acid experiments that there is an increase of per cent. of turnings from the high acid concentration end over the low concentration acid end in the order named. Thus the hydrochloric acid again has the greatest intensity in avoidance of the high acid concentration end. These points are shown by the experiments with *propinquus* although the acetic acid experiments were of longer duration and were performed at a lower temperature than the hydrochloric acid experiments. The conclusion that intensity of negative reaction is directly proportional to the concentration of H ions can only be suggested, as sufficient data to warrant a definite conclusion are wanting.

The above suggestion might receive objection on the ground that there may be specific differences in the effect of the three acids used. Such an objection is supported by the fact that *propinquus*, in the low temperature experiment, is intoxicated by carbon dioxide and not by acetic acid. This apparent difference may, however, be explained on the ground that the carbon dioxide, since it diffuses more rapidly than the acetic acid, really produces a higher hydrogen ion concentration in the blood of the crayfishes than does the acetic acid, in spite of the fact that the latter acid is more highly ionized. The carbon dioxide would also tend to increase the carbon dioxide in the animal's blood by preventing the escape of the supply of this gas that is constantly being given off by the tissues of the animal. The acetic acid and hydrochloric acids would not offer any such hindrance to the diffusion of the internal gas into the water, and would not, therefore, be as detrimental as the carbon dioxide.

The periodicity and final cessation of invasion of the high concentration end of the experimental tank is a modification of behavior that may be brought about by increase in sensitiveness on the part of the crayfishes, or by a more rapid reaction to the same sensation. In the one case the cause is physiological, in the other the explanation must be psychological. If the

modification is psychological then the animals must respond from associated memory. Shelford and Allee ('13, '14) have pointed out that it is hard to locate the things associated. Besides association formation is usually very slow for Yerkes ('08) states that 50 to 100 trials are necessary for the crayfish to form a perfect association in a simple labyrinth. The same slowness of modification would be expected of association due to a stimulus unless the sensitiveness of the animals was in some way progressively increased.

The modification is rapid, the number of invasions being sometimes but one before complete avoidance of the high end followed. This modification is probably due, as Shelford and Allee ('13) have pointed out, to increased sensitiveness on the part of the crayfishes and as they have further suggested, the greater sensitiveness may be the result of an increase in the hydrogen ion content of the blood of the animals.

In the cases where *propinquus* came to rest upon the screen in the low end and remained there for the rest of the experiment, the reaction may be considered the climax of the behavior modification, especially since the animals made this reaction more quickly in the presence of high total concentrations of acid than in the low. These points are shown by *propinquus*, Chart I., Expts. 5, 14. The explanation as to why the animals came to rest at all after being made more sensitive by the acid is not clear, but probably a combination of factors, one of which is thigmotaxis, were acting.

Shelford and Allee ('13) suggest carbon dioxide as a factor in determining the distribution of fishes and that the same may be true for crayfishes is suggested by the foregoing experiments. Crayfishes react to very weak concentrations of carbon dioxide and acetic acid and they were not overcome by the carbon dioxide except in concentrations higher than usually appear in natural waters. There seems also to be a correlation of the specific reactions of the two species with their respective habits. *Propinquus* is a pond form and its reactions were directive, while *virilis*, a rapid stream form, gave reactions which were much less directive. In natural waters carbon dioxide would be encountered in rather high concentrations by *propinquus* and that this species may react to these concentrations to its own advan-

tage is indicated by the experiments heretofore described. *Virilis*, however, in its stream habitat would seldom encounter carbon dioxide concentrations of anything more than a very low degree and thus we find that this species does not react definitely to the gas. *Virilis*, then probably, while sensitive to carbon dioxide, orients itself in its environment by reactions to some other factor or factors. *Immunis* and *diogenes* are pond forms and are less sensitive to the acids but both avoid high concentrations. *Diogenes* although shown to be more sensitive than *immunis* orients less definitely as is shown by the per cent. of turnings from the high and low concentration acid ends. The specificities are again in coördination with the habitats of the species, *immunis* being a pond mud loving form, remaining in burrows only at times while *diogenes* remains almost wholly in burrows.

## Summary.

1. Crayfishes sense the increase in carbon dioxide and acetic and hydrochloric acids in a gradient.

2. Both *propinquus* and *virilis* are intoxicated by carbon dioxide; *virilis* is also intoxicated by acetic acid but to a lesser degree.

3. The four species are susceptible to high concentrations of carbon dioxide and when subjected to high concentrations die in the following order, *virilis*, *propinquus*, *diogenes* and *immunis*.

4. *Propinquus* reacts negatively to the higher concentrations of carbon dioxide in a gradient, but when the total amount of acid present is large, the negative reaction may be interfered with by the direct detrimental effect of the acid.

5. *Virilis* reacts less definitely to the higher concentrations of carbon dioxide in a gradient than does *propinquus*. This is true whether the total concentration of the acid is large or small.

6. Both *diogenes* and *immunis* react more or less irregular to carbon dioxide due possibly to the lesser sensitiveness of these two species to this acid.

7. Both *propinquus* and *virilis* react negatively to the higher concentrations of acetic acid in gradients of this acid; *propinquus* reacts definitely in the presence of both high and low total concentrations; *virilis* reacts definitely to low total concentrations,

but not so definitely to high total concentrations; *diogenes* reacts irregular and less intense than the first two species while *immunis* reacts more definitely but with low intensity.

8. All four species react more strongly to hydrochloric acid than acetic acid and more strongly to acetic acid than carbon dioxide.

9. The intensity of avoiding reactions of all species to all acids tested as is shown by turnings only are in the following order; *propinquus*, *virilis*, *immunis* and *diogenes*.

10. The intensity of avoiding reactions of all four species varies directly as the total concentrations of the acids, and probably directly as the hydrogen ion concentration.

11. Rapid modification of behavior is shown by all four species. This modification may be due to the increased sensitiveness on the part of the animals, the increased sensitiveness being the result of higher ion concentration in the animal's blood.

12. The specific reactions of the crayfishes in gradients of carbon dioxide may be correlated with their habitats.

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# BREEDING HABITS OF THE HETERONEREIS FORM OF PLATYNEREIS MEGALOPS AT WOODS HOLE, MASS.

E. E. JUST.

Verrill ('73) first described *Platynereis megalops* figuring in his "Report" a male of the heteronereid phase. Later ('79) he figured the nereis-form and the female of the heteronereis-form changing the name he first gave, *Nectonereis megalops*, to *Nereis megalops*. Andrews, who ('91) had discovered the egg *Nereis limbata*, in a paper on the eyes of annelids speaks of the worm as *Nereis alacris*. I am indebted to Dr. J. Percy Moore who identified the animal as *Platynereis megalops*, Verrill. The belief seems to prevail that in the study of the cell lineage of *Nereis* ('92) Wilson indiscriminately used the males and females of *Nereis* and *Platynereis*. But this belief is by no means founded on any statement in Wilson's paper. Bonnevie ('08) has perhaps strengthened popular misconception through her descriptions of the "two varieties" of *Nereis limbata* at Woods Hole.

## I. Swarming Habits.

The swarming of *Platynereis* is closely similar to that of *Nereis* (cf. Lillie and Just). There seems to be some variations as noted below. The behavior shows as that of many other forms a definite lunar periodicity: the sea-urchins (Tennent), the Japanese palolo (Izuka), the Pacific palolo (Woodworth and others), the Atlantic palolo (Mayer), *Amphitrite* (Scott), *Nereis dumerilii* (Hempelmann), etc.

Observations were made during the seasons of 1911, 1912 and 1913 at the Marine Biological Laboratory, Woods Hole, Mass. The swarming habits of *Platynereis* have not been worked out as fully as have been those of *Nereis limbata* (Lillie and Just, '13). In the first place during 1911 and 1912 attention was focused mainly on the swarming habits of *Nereis;* moreover, at all times the primary object in the collecting of *Platynereis* was for

experimental study. Strict watch, however, was kept on these worms throughout the summers named. This is especially true for the summer of 1913; during June, July, and August I went out every night, giving attention wholly to *Platynereis* swarming.

The animals on swarming nights swim near the surface of the sea, the males invariably appearing first, the females later. The females rarely exceed fifteen, and indeed on some nights no females swim, while the number of males may be very large. Verrill ('73) says that the worms swim at noon. I have never noted this.[1] Hempelmann ('11) might lead one to think that *Nereis dumerilii* swarms early in the morning. I looked for this during August, 1913, but did not find *Platynereis* swarming before or at sunrise. The evening swarm may last two hours.

The small reddish males swim with great rapidity in an ever more narrowing circle within the patch of light thrown by the observer's lantern until the swarm is at its height. Here and there often at a greater depth than the males swims with slow and even laborious movements, the larger female, pale yellow in color with a thin dorsal line of green—the remnant of the empty gut. One cannot but suspect that the sex ratio in some way depends on the rate of movement: the females are easy prey for fish, the males must easily escape their enemies. The sex ratio of the captured animals must be also influenced by the fact that the females tend to keep further below the surface than the males. This is true of *Autolytus* to a marked degree as I have repeatedly observed. (So too, Andrews, '92, and Mensch.) Verrill, however, says of *Nereis limbata* that in their burrows "there are few males in proportion to the females"—as in the case of *Platynereis*, the reverse is true of these worms during swarming.

As the male comes in the vicinity of female he swims very rapidly in spirals tangential to the surface. They swim together and after copulation and egg-laying, the female slowly sinks from view.

The swarming occurs nightly throughout the months of July and August during the dark of the moon. From new moon to full moon, whether there be moonlight or not the animals do not swarm. Only mature animals swarm.

[1] In July, 1914, I found spent males swimming during the day.

I have never taken this Heteronereid at Woods Hole earlier than June 29. In 1911 I remained at Woods Hole until September 18; I took no worms after August 24. For 1913, August 19 is the date of last capture.

The following tables selected from data of 1911, 1912 and 1913 give some idea of the lunar periodicity of the swarming:

TABLE I. 1911.

(Date of first capture, July 20.)

| Moon Phase. | Date. | Number of Females. | Number of Males. |
|---|---|---|---|
| Full moon | August 8 | 0 | 0 |
| | 9 | 0 | 0 |
| | 10 | 0 | 0 |
| | 11 | 0 | 0 |
| | 12 | 0 | 0 |
| | 13 | 0 | 0 |
| | 14 | 0 | 0 |
| | 15 | 0 | 0 |
| | 16 | 1 | 6 |
| Third Quarter | 17 | 0 | 0 |
| | 18 | 0 | 0 |
| | 19 | 0 | 0 |
| | 20 | 0 | 0 |
| | 21 | 4 | 5 |
| | 22 | 6 | 6 |
| New Moon | 23 | 8 | 8 |
| | 24 | 10 | 10 |
| | 25 | 0 | 0 |
| | 26 | 0 | 0 |
| | 27 | 0 | 0 |
| | 28 | 0 | 0 |

Comparison with *Nereis* shows in the first place that the number of worms swarming is not so great. It was found, for instance, in collecting *Nereis* to be practically impossible to make an accurate estimate of the number of males; for that reason a record was kept of the females only. On two or three nights only did I find it impossible to estimate the number of *Platynereis* males swarming; on other evenings it was easily possible to count them. The swarm of males on the evening of August 11, 1912, was wonderful. For a few minutes the sea was alive with thousands of the rapidly swimming Heteronereids. In 1913 there was a similar swarm of females, but in no such numbers. As in the case of *Nereis* the collections were made in one place during the three years.

The season, moreover, appears to be shorter than that of

## TABLE II. 1912.

| Moon Phase. | Date. | Number of Females. | Number of Males. |
|---|---|---|---|
| | June 4 to July 2 | None | None |
| | July 3 | 0 | 1 |
| | 4 | 0 | 0 |
| | 5 | 0 | 0 |
| | 6 | 0 | 0 |
| Third Quarter | July 7 | 0 | 0 |
| | 8 | 0 | 0 |
| | 9 | 0 | 0 |
| | 10 | 1 | 3 |
| | 11 | 3 | 3 |
| | 12 | 3 | 3 |
| | 13 | 0 | 2 |
| New Moon | 14 | 2 | 2 |
| | 15 | 0 | 2 |
| | 16 | 0 | 1 |
| | 17 | 0 | 0 |
| | 18 | 0 | 0 |
| | 19 | 0 | 0 |
| | 20 | 0 | 0 |
| First Quarter | 21 | 0 | 0 |
| | 22 | 0 | 0 |
| | 23 | 0 | 0 |
| | 24 | 0 | 0 |
| | 25 | 2 | 1 |
| | 26 | 1 | 0 |
| | 27 | 0 | 1 |
| Full moon | 28 | 0 | 0 |
| | 29 | 0 | 0 |
| | 30 | 15 | 30 |
| | 31 | 2 | 30 |
| | August 1 | 4 | 30 |
| | 2 | 3 | 30 |
| | 3 | 0 | 20 |
| | 4 | 0 | 0 |
| Third Quarter | 5 | 8 | 20 |
| | 6 | 14 | 20 |
| | 7 | 0 | 0 |
| | 8 | 12 | 10 |
| | 9 | 10 | 50 |
| | 10 | 12 | 100's |
| | 11 | 15 | 1000's |
| New Moon | 12 | 18 | 30 |
| | 13 | 0 | 0 |
| | 14 | 0 | 0 |
| | 15 | 3 | 6 |
| | 16 | 0 | 0 |
| | 17 | 0 | 0 |
| | 18 | 0 | 0 |
| First Quarter | 19 | 0 | 0 |
| | 20 | 0 | 0 |
| | 21 | 0 | 0 |
| | 22 | 0 | 0 |
| | 23 | 0 | 0 |
| | 24 | 0 | 0 |
| | 25 | 0 | 0 |
| | 26 | 0 | 0 |

TABLE II. 1912.—*Continued.*

| Moon Phase. | Date. | Number of Females. | Number of Males. |
|---|---|---|---|
| Full moon | 27 | 0 | 1 |
| | 28 | 0 | 1 |
| | 29 | 0 | 0 |
| | 30 | 0 | 0 |
| | 31 | 0 | 0 |

TABLE III. 1913.

| Moon Phase. | Date. | Number of Females. | Number of Males. |
|---|---|---|---|
| | June 13–28 | None | None |
| | 29 | 0 | 1 |
| | 30 | 0 | 0 |
| | July 2 | 0 | 1 |
| | | None | None |
| | 17 | 0 | 1 |
| Full moon | 18 | 0 | 10 |
| | 19 | 0 | 30 |
| | 20 | 0 | 20 |
| | 21 | 1 | 25 |
| | 22 | 0 | 10 |
| | 23 | 0 | 8 |
| | 24 | 2 | 4 |
| | 25 | 50 | 30 |
| Third Quarter | 26 | 2 | 4 |
| | 27 | 5 | 3 |
| | 28 | 16 | 20 |
| | 29 | 5 | 8 |
| | 30 | 20 | 16 |
| | 31 | 21 | 8 |
| | August 1 | 6 | 6 |
| New Moon | 2 | 1 | 20 |
| | 3 | 4 | 25 |
| | 4 | 0 | 16 |
| | 5 | 0 | 0 |
| | 6 | 0 | 1 |
| | 7 | 0 | 0 |
| First Quarter | 8 | 0 | 0 |
| | 9 | 0 | 0 |
| | 10 | 0 | 0 |
| | 11 | 0 | 0 |
| | 12 | 0 | 0 |
| | 13 | 0 | 0 |
| | 14 | 0 | 0 |
| | 15 | 0 | 0 |
| Full Moon | 16 | 0 | 2 |
| | 17 | 1 | 0 |
| | 18 | 0 | 1 |
| | 19 | 0 | 2 |
| | 20 | 0 | 0 |
| | 21 | 0 | 0 |

*Nereis.* In this my observations approximate those of Verrill. Also, the yearly swarming shows more variations than that of

*Nereis*. This is strikingly brought out by a study of the tables—especially when one recalls that I gave attention wholly to *Platynereis* for the year 1913. Curves of the runs of *Platynereis* would show that the heights tend to fall in with those of *Nereis*. The lunar periodicity is therefore more like that of *Nereis limbata* than that of *N. dumerilii* which in some respects *Platynereis* resembles.

## II. Egg-Laying.

Males and females caught with a hand-net in the evening at the surface of the water and kept in separate dishes may be studied in the laboratory. If a male be transferred with a female to a dish of clean sea-water, the phenomena observed in the sea may be readily followed. The female packed eggs discernible through her pale thin body wall swims slowly in a straight line; or, with head bent at right angles to the body describes a circle of which the head is the center. The male swims in spirals tangential to the surface of the water. Soon his spirals are along the course of the female, her body finally becoming the long axis of his helical body. He entwines the female through this performance and straightens out, thus clutching her in the twist of his body. If this embrace be in the posterior region of the female's body, the male loosens slightly and pulls himself along the female's body. The task appears to be exacting. Often I have observed a rather small male that had worked himself forward after having grasped an unusually large female near the anal segment fall apparently too exhausted to complete the courtship. As the male slips along forward over the female, he lashes his tail back and forth. The female bends her head as if seeking the tail. If the female keep her body in a straight line, the male must move anteriorly until he entwines her body in the pharyngeal region. He now forms a coil around her head of which his tail is the apex. He thrusts his tail down into the coil of his own body and so into the waiting jaws of the female. The female is quiescent throughout. About six seconds after the female has received the anal segment of the male, the animals separate and eggs stream from the posterior segments of the female. The male may be held for a time by the female; if so he swims around, dragging her. I believe that the eggs

escape, not through gonopores or the like, but through lesions of the body wall (cf. Scott.) Eggs escape from three or more posterior segments, occasionally from anterior segments. If escape by way of the posterior segments be experimentally inhibited, or if the female be slightly disturbed, the eggs seem to burst through the body wall at segments more anterior than otherwise. Females killed at the moment of oviposition show tears in the body wall.

After oviposition—and the whole process just described is in general the event of ten seconds—the female sinks to the bottom of the dish, a mere shred. In the laboratory placed in a little water it remains an irritable sticky mass for a time—incapable of exciting fresh males and finally dies, greatly shrivelled and blackened. Often, however, if flooded with fresh sea water it revives, expands to previous size, and swims around actively, almost perfectly transparent. I have kept these spent females alive for several hours. Since there are no sexual segments as in some annelids, but the whole body is little more than a locomotor ovary, it seems safe to assume that this egg-laying marks the end of the worm's existence.

Both animals must be in healthy condition for this behavior. Active males sometimes grasp females which because of rough handling in capturing are doubtless weak and fail to respond. The active males on the other hand are not very hardy: in the laboratory they rarely live twenty-four hours; one experiment made in 1913, failed to show any difference in the vitality of spent and unspent males. Normal females when placed in dishes with males fail to complete the courtship if the vitality of the male as by rough handling be impaired. Males and females may be kept in the same dish until death; if there be no courtship, there is no oviposition. Female *Platyneris* and male *Nereis* show no excitement when in the same dish, so male *Platynereis* and female *Nereis*. The male *Platynereis* ordinarily will embrace only an unspent female *Platynereis*. But on one occasion (July 23, 1913) all (8) males captured in turn and repeatedly embraced a *Nereis virens* eight inches long whose posterior segments had been lost. Once only I saw a male clutch a female which had extruded part of her eggs after a previous courtship.

The animals will go through this courtship when placed in a

very dense suspension of India ink in sea-water; or total darkness. The reaction, therefore, cannot be due to sight. It is more likely due to some chemical emanation from the gravid female only since the spent female is not attractive to the male (Cf. F. R. Lillie on *Nereis*, '12, '13.)

A male *Platynereis* will embrace at least four females. On the evening of August 24, 1911, for instance, I put a male and a female in a dish. They swam around for a time, then the male wrapped himself about the female just back of the head, he let go, uncoiled himself, his tail remaining in the female's mouth. Immediately after release, he was placed with a second female; a minute later he induced oviposition. After intervals of five minutes he embraced a third and fourth female. In all cases the worms shed eggs. The male placed in fresh sea-water with an active female after an hour (11 P.M., about two hours in the laboratory after capture) failed to make a fifth clutch. Other males embraced two females. During 1912 and 1913 these observations were verified.

If after this egg laying behavior, both animals be removed from the dish or if the eggs be pipetted off as laid the eggs develop and normal swimming larvæ much like those of *Nereis limbata* result. If at the moment of her release by the male the female be put in a dish of clean fresh sea-water, eggs will stream out and subsequently develop.

In all these cases sperm are attached to the vitelline membrane within a hull of jelly which has been secreted through the breakdown of the cortical protoplasm of the egg. As in *Nereis* this jelly formation begins at the moment that the sperm touches the membrane. In *Platynereis* it is easily demonstrated that the inseminated eggs have this jelly when laid. Mechanical pressure either by the male, experimentally, or otherwise, as has been repeatedly demonstrated, will not induce oviposition. Mere clutching however recurrent—even by more than one male is not sufficient stimulus for oviposition. The head of the worm may be crushed—eggs will not escape; if she be cut in two, a few eggs escape. Only after thorough drying on filter paper or on sheer dry linen will the eggs burst through the body wall. If the female be finely minced in sea-water practically all the eggs may

be procured. But eggs got in this way do not develop after insemination; they will not fertilize in sea-water. I have sections of uninseminated eggs killed after having remained upwards of two hours in sea-water; the cortical layer and the germinal vesicle are intact. (So *Nereis*.)

Eggs removed from worms after clutching only have the appearance of eggs from unembraced females—no sperm attached, subsequently no trace of development. Sperm are not found on the female's body (*e. g.*, hypodermic impregnation: cf. Whitman, Gardiner, etc.) or near the anus at the time of egg extrusion.

It appears, therefore, that mechanical stimulus is not sufficient to excite oviposition or sperm shedding. The eggs are not laid during or after the embrace nor are sperm shed unless the male's tail has been in the female's jaws. This, then, is a case of copulation followed by internal insemination. And indeed, the very elaborate and precise behavior indicates this. The sperm swallowed by the female inseminate the eggs in the body cavity, oviposition following immediately.

In 1911 gravid females before and after copulation were killed in Meves fluid but proved too refractory for cutting; in 1912, special precautions were taken. The following fixatives were used: Bouin, Gilson, 10 per cent. formalin, and Hennings mixture. With these mixtures the yolk and oil of the eggs are dissolved out, but the chitin of the jaws still makes the procuring of good sections difficult. In 1912 I thought that I had solved the difficulty when after experiments with various agents I procured with KCl, and KCN in sea-water eversion of the pharynx. But in 1913, these methods gave very indifferent results. Dissection of the jaws gave almost negative results. My best sections are those of July, 1912, killed in Gilson, Series A; those of August, 1912, killed in formalin, Series B, and those of 1913 kept in formalin for five months.

Sections of gravid females killed before courtship show no sperm in the body cavity. Sections of gravid females just after copulation show sperm among the antennæ, in the mouth, in the pharynx, and in the body cavity. The sperm may be traced, therefore, entering the mouth, passing down the pharynx whence they escape through lesions in the pharyngeal wall to the cœlom.

They may be found also attached to the vitelline membrane of the eggs. If one minces a male, one procures not only sperm but large numbers of corpuscles. Apparently, these are not injected into the female's body (cf. Scott on *Amphitrite*).

Since the mechanical pressure of the male, though often repeated, is not sufficient stimulus for egg-laying, it may be assumed that either the sperm or some secretion with, or of them stimulates in the female movements which bring about oviposition. In some cases males after having induced oviposition in two or three females cause egg-laying in a third or fourth as noted above. A slight amount of this substance, therefore if such there be in addition to the sperm themselves, is sufficient to initiate egg-laying. The injected substance, on the same ground could scarcely exert sufficient pressure to stimulate oviposition.

I had projected for 1913 various experiments to determine this point. The first experiment on the list, however, was clear enough to warrant abandoning the others. I put a female in a dish with no water. If a drop of sea-water be put on her head there is no response. Only complete drying causes breakdown of body wall. If instead of pure sea-water the minced female be added there is no response. But if a drop of minced male be added oviposition follows. This observation was made several times.

The following protocol from notes of the night of July 25, 1913, is typical:

*Experiment.*—Six males cut up in water adherent to their bodies (*i. e.*, not dried). Dried female put in this sperm suspension. No oviposition. A second female placed in the sperm suspension; and a third. No oviposition.

2. Three males cut up in three drops of sea-water. Two successive females used. No oviposition.

3. Six dried males cut up. Two dried females placed with heads in the sperm suspension. Eggs laid. Next day: trochophores.

4. Three males cut up in two drops of sea-water. Two dried females placed with heads in the sperm suspension. (Both females later copulated with males and laid eggs.) No eggs laid.

Oviposition, then, is clearly brought on through the ingesting of sperm with very little sea-water.

*Nereis diversicolor* O. F. Muller gives birth to living young. *Autolytus* (Agassiz) carries its larvæ in a brood pouch. In both of these forms there is probably internal insemination. Eisig

has described copulation in an annelid, *Capitella*. *Platynereis* is of interest in that oviposition so quickly follows copulation.

## III. The Nereis Form of Platynereis Megalops.

As in the case of *Nereis* an attempt has been made to rear the larvæ of *Platynereis*. Best results were obtained during 1913. A table was kept of the development of the young worms and their characteristics noted. They closely resemble the larvæ of *Nereis dumerilii* described by Hempelmann which he obtained from his cultures. Some of my worms aged six months measured four centimeters. It is hoped that a study of these forms will give a clue to the swarming habit.

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# ON THE STRUCTURE OF THE INNER EAR IN TWO PRIMITIVE REPTILES.

E. C. CASE.

In 1885 Cope[1] described the structure of the brain and the inner ear of one of the cotylosaurian reptiles, *Diadectes sp.* In this paper he figured the structure of the canals and concluded as follows: "The result of this examination into the structure of the auditory organs in the Diadectidæ may be stated as follows: The semicircular canals have the structure common to all the Gnathostomatous Chordata. The internal wall of the vestibule remains unossified as in many of the fishes and a few batrachians. There is no rudiment of the cochlea, but the vestibule is produced outward and upward to the fenestra ovalis in a way unknown in any other family of the vertebrates."

Fig. 1 shows the arrangement of the semicircular canals as given by Cope.

Among the many specimens collected from the Brier Creek Bone-bed by the University of Michigan expedition to Archer County, Texas, in the summer of 1913 there are several complete and nearly complete basi-cranial regions of the Permian or Permo-Carboniferous reptiles, *Dimetrodon* and *Edaphosaurus*. The structure of the basi-cranial region in these forms has already been described by the author,[2] but the recovery of this new material makes it possible to describe the condition of the ear cavity.

The specimen of *Edaphosaurus*, No. 3446 University of Michigan, probably belongs to the species *cruciger* of Cope. The bones of the ear region are undistorted and the cavity shares in the perfection of preservation. This is shown by the complete correspondence of the two sides and by the similarity of the cavity to that in less perfectly preserved specimens. If the co-

[1] Cope, *Proc. Am. Phil. Soc.*, p. 234, 1885.

[2] Case, Publication 55, Carnegie Institute of Washington, p. 98, 1907. Williston and Case, Publication 181, Carnegie Institution of Washington, p. 81, 1912.

ossified elements which shelter the inner ear be held in the normal position, as shown in Fig. 2, it will be seen that there is the trace of a groove running apparently horizontally from without inward; at the inner upper corner of the cavity it joins the trace of a second groove running nearly vertically. These are the marks of the anterior and posterior semicircular canals and correspond to the horizontal antero-posterior and the vertical antero-

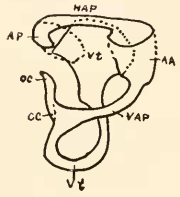



FIG. 1.

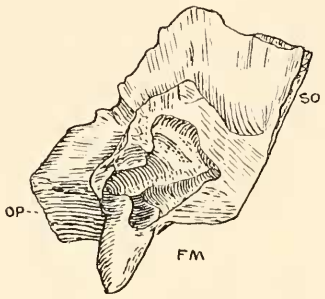



FIG. 2.

FIG. 1. Outline of the canals of the inner ear of *Diadectes* sp. From Cope. *hap*, horizontal antero-posterior canal; *aa*, anterior ampulla; *vap*, vertical antero-posterior canal; *vt*, vertical transverse canal; *ap*, posterior ampulla; *cc*, canalis communis of the vertical antero-posterior and the vertical transverse canals; *oc.os*, commune of the same.

FIG. 2. Inner view of the right otic region of *Edaphosaurus*, showing the cavity of the ear. *So*, supraoccipital; *op*, opisthotic; *fm*, foramen magnum. × 1.

posterior canals of Cope's figure. Where the two canals meet there is a projection upward and forward of the cavity which probably lodged a rudiment of the apex of the sinus utriculus superior (terminology of Retzius). At the outer ends of the canal there is evidence of slight enlargements which lodged the anterior and posterior ampullæ. Just below the point of union of the two canals there is an elevation of the cavity indicating that the sinus utriculus posterior was inclined inward and forward. The cavity for the utriculus is relatively large and higher than wide. On the outer side of the broken surface of the cavity, nearly opposite the middle point there is a slight excavation indicating the position of the external canal. At the lower end of the cavity there are two extensions, one running into the paroccipital (opisthotic) bone and parallel to its axis, this lodged the

lagena and shows no evidence of any curvature; the second extension is smaller and connected with the lagena cavity, it can only be for a considerable remnant of the sacculus.

The inner wall of the otic cavity was incomplete and the fenestra ovalis opened about opposite the upper end of the lagena.

The second specimen, No. 3447 University of Michigan, is that of one of the smaller but undeterminable species of *Dimetrodon*. It is as well preserved as that of the *Edaphosaurus*, both sides being present and identical in appearance and the details of the structure further verified by other specimens. The general arrangement of the canals and cavities is the same as in *Edaphosaurus* but it is smaller. The evidence for the presence of a

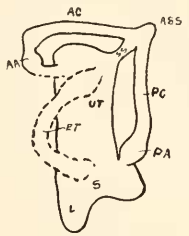



FIG. 3.

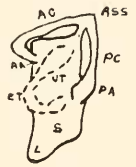



FIG. 4.

FIG. 3. Outline of the canals of the right side in *Edaphosaurus*. *ac*, anterior canal; *ass*, apex of the sinus urticule posterior; *pc*, posterior canal; *pa*, posterior ampulla; *s*, sacculus; *l*, lagena; *et*, external canal; *ut*, utriculus; *aa*, anterior ampulla. × 2.

FIG. 4. Outline of the canals of the right side in *Dimetrodon* sp. Lettering the same as in Fig. 3. × 2.

apex of the sinus utriculus superior is less pronounced than in the first specimen but there can be no doubt of its presence. The two lower cavities, for the lagena and the sacculus, are narrower and in less open connection. The arrangement of the canals is shown in Fig. 4.

The author is unable to bring the structure as here made out into adjustment with the figures given by Cope for *Diadectes*. In comparing the structure of the inner ear of these primitive forms with that of modern reptiles certain archaic characters are recognized. The presence of an apex to the sinus utriculus superior is notably primitive, Retzius shows it as present in certain fishes but it is not noted in the amphibians and reptiles.

The straight lagena and the relatively large remnant of the sacculus is also primitive; a large sacculus occurs in the Amphibia Ecaudata and in some lizards, as *Lacerta viridis*. The canals are entirely within the otic cavity and do not penetrate any of the adjacent bones. The lagena of the crocodile is quite elongate and the soft structures are said to show something of a twisting, the lagena of these forms is relatively shorter and broader than that of the crocodile but, of course, nothing can be said of the soft parts. The cavity of the inner ear does not exactly reproduce the membranous ear as the soft parts are separated from the wall by the perilymph but the author believes that the figures given represent a very fair approximation to the true form and proportions of the membranous ear.

# PRELIMINARY REPORT OF CROSSING TWO HEMIPTEROUS SPECIES, WITH REFERENCE TO THE INHERITANCE OF A SECOND EXCLUSIVELY MALE CHARACTER.

KATHARINE FOOT AND E. C. STROBELL.
OXFORD.

Attention of cytologists has been centered during the past few years on the chromosome theory that claims to offer an explanation of sex-determination—a theory due to the discovery of certain morphological differences in the chromosomes of the males and females of many species. This discovery is responsible for the chromosome hypothesis of sex-determination—an hypothesis that awaits the test of experiment.

Recently (Foot and Strobell, '13) we published the results of some cross-breeding experiments undertaken with the aim of testing the above mentioned hypothesis that the factors determining sex are carried and distributed by definite chromosomes. The character selected to test this hypothesis was an exclusively male character in *Euschistus variolarius*—a distinct dark spot which is present on the genital segment of the male only, and we claimed that the method of the transmission of this spot should be an index of the method of transmission of the entire male genital segment. This exclusively male character—the genital spot—is a distinguishing feature of *Euschistus variolarius*, but is absent in *E. servus*. We therefore selected these two species as well adapted to test the function of the so-called sex-chromosomes in the transmission of this exclusively male character. Our claim that the method of transmission of the genital spot may be interpreted as an index of the transmission of the male reproductive organs themselves has not been accepted by two critics (Morgan and Doncaster) who have recently referred to our results. In reply to the criticism of these two advocates of the chromosome theory of sex-determination, we quote the following from a paper now in press (Foot and Strobell, '14*b*).

"After this paper was sent to press a notice of our results appeared in the following publications: 'Heredity and Sex,' Morgan ('13), and 'Chromosomes, Heredity and Sex,' Doncaster, *Q. J. M. Sci.*, Vol. LIX. ('14).

The latter disposes of our results in a footnote, as data irrelevant to a paper entitled "Chromosomes, Heredity and Sex—A Review of the Present State of the Evidence with Regard to the Material Basis of Heredity, Transmission and Sex-Determination."

From his report of the evidence he draws the following conclusion:

"The facts of sex-limited[1] transmission thus support the hypothesis that both ordinary Mendelian factors and the sex-determining factor or factors are borne by chromosomes," p. 511, and in the above-mentioned footnote he adds: "The recently published work of Foot and Strobell cannot be used as an argument against this proposition. They have shown (as was previously known in birds and moths) that a secondary sexual character in Hemiptera can be transmitted through the sex that does not show it; but the character was not sex-limited[1] in transmission; their results, therefore, have no bearing in the present discussion." As opposed to this decision we claim that the very fact that the genital spot is not linked with one of the so-called sex chromosomes is a point that calls for a satisfactory explanation by those who believe in sex-determining chromosomes, and our results cannot be cancelled by a dogmatic assertion that they have no bearing on the subject.

Morgan treats the facts with more consideration and attempts to give an explanation of them, though his explanation appears to us more as an attempt to excuse the facts than to explain them. Part of his explanation is merely a restatement of our conclusions, and the remainder is not in harmony with the facts.

We concluded that our results demonstrate that the spot can be transmitted without the X- or the Y-chromosome and Morgan accepts this as follows, "these results may be explained on the assumption *that the factors lie in other chromosomes than the sex-chromosomes.*"[2]

[1] Sex-limited is used by Doncaster in the sense that sex-linked is used by Morgan.

[2] The italics are ours.

We concluded that if one assumes (for the sake of the argument) that the spot factors are in a diploid pair of chromosomes, it becomes necessary to assume other factors outside the chromosomes. We called such hypothetical factors "inhibiting factors" and we said of them: "We are forced to admit that inhibiting factors—whatever they are—must be located outside the chromosomes, in the region of pure hypothesis."

Morgan appears to accept this, calling such hypothetical factors "*things in the cell*," and symbolizing them as A. B. C. He says "the result (or character) that a factor produces depends on its relation to *other things in the cell* (here A. B. C.)," and he adds, "We are dealing, then, not with the relation of X to S *alone*, but this relation in turn depends on the proportion of both X and S to A. B. C."

In the above-quoted paragraph he includes in his explanation the assumption of a relation between the spot factors and the X-chromosome, and this we believe is a part of his explanation which is not sustained by the facts. The spot can be transmitted directly from the male to his male offspring—and therefore this must be by the male-producing spermatozoön—(if there is such a thing) and the so-called male-producing spermatozoön has no X-chromosome. It is impossible to believe that in such cases the inheritance of the spot is dependent upon the relation of the spot factors of the sperm to the X-chromosome in the egg, especially if the cross is made with a pure *servus* egg. Morgan evidently thinks this is possible, however, for in his explanatory diagram he illustrates a cross between *E. servus* ♀ × *E. variolarius* ♂, a cross which we explained we were unable to attempt on account of scarcity of material.

His diagram, if assumed to be an explanation based on the facts of our experiments, is further in error in its illustration of the $F_1$ hybrids. In his simple Mendelian scheme all the $F_1$ hybrids are illustrated as typical heterozygotes and the fact is ignored that two out of the eleven of our $F_1$ hybrids are like *servus* in having no spot. If such a modification of the spot can be caused by "*other things in the cell*," it would seem that merely calling these "A. B. C." is no explanation of the results.

Morgan excuses his attempted explanation on the ground

that we have failed to explain our results. We make no apology for this. We believe the duty of the scientist is to curb the natural temptation to force an explanation of individual results, for science to-day is overburdened by premature and undigested generalizations. We would aim rather to follow the example of those scientists who are willing patiently and conscientiously to collect data sustained by the hope that some day the facts may be of value.

Both Morgan and Doncaster class the genital spot of *variolarius* with the secondary sexual characters of authors and they therefore interpret our results as not having the bearing on the theories of sex-determination which we claim for them. Now our claim has been that the genital spot of *variolarius* is an integral part of the male genital segment—the structure of the female genital segment being such that the spot could not be present in this segment without changing the form of the segment itself—and we have claimed that therefore a study of the transmission of the genital spot should give a trustworthy indication of the method of transmission of the entire genital segment.

This claim that the method of transmission of the genital spot should be an index of the method of transmission of the genital organs of the male, has been completely justified by further work on these hybrids.

In the present paper we shall report the results from the study of the transmission of a second exclusively male character, and it seems to us that these results cannot be set aside as having no bearing on "Sex and Heredity," for this second exclusively male character is the male genital organ itself—the intromittent organ. The genetic results from our study of the genital spot of *variolarius* may be open to the criticism that as the spot is "not directly connected with the act of reproduction" it should be classed with the secondary sexual characters; but the intromittent organ is certainly free from such criticism and can be justly classed as a primary sexual character. In view of the fact that our results from the study of the transmission of the *variolarius* spot have been set aside on the ground that the spot is a secondary sexual character and therefore has no bearing on the problem of the determination of sex, it is necessary first

to establish the claim that the intromittent organ can be classed with the primary and not the secondary sexual characters. This apparently ought not to be difficult, but a difficulty does arise owing to the fact that recent authors who have discussed secondary sexual characters have avoided defining them and have neglected to state wherein they are to be distinguished from the primary sexual characters.

According to Darwin '59 Hunter defines secondary sexual characters as follows:

"The term, secondary sexual characters, used by Hunter, applies to characters which are attached to one sex; but are not directly connected with the act of reproduction."

Darwin '86 adopts Hunter's classification of primary and secondary sexual characters; but shows that even such an apparently clear cut definition encounters difficulties. He says:[1]

"With animals which have their sexes separated, the males necessarily differ from the females in their organs of reproduction; *and these afford the primary sexual characters.* But the sexes often differ in what Hunter has called secondary sexual characters, which are *not directly connected with the act of reproduction;* for instance, in the male possessing certain organs of sense or locomotion, of which the female is quite destitute, or in having them more highly-developed, in order that he may readily find or reach her; or again, in the male having special organs of prehension so as to hold her securely. These latter organs of infinitely diversified kinds graduate into, and in some cases can hardly be distinguished from, those which are commonly ranked as primary, such as the complex appendages at the apex of the abdomen in male insects. Unless indeed we confine the term 'primary' to the reproductive glands, it is scarcely possible to decide, as far as the organs of prehension are concerned, which ought to be called primary and which secondary" (p. 253).

Morgan '13 also appears to accept Hunter's classification, for in his rather full list of secondary sexual characters he includes none that are "directly connected with the act of reproduction." He opens his discussion of secondary sexual characters as follows:

[1] The italics are ours.

"The Secondary Sexual Characters."

"In the most highly evolved stages in the evolution of sex a new kind of character makes its appearance. This is the *secondary sexual character*. In most cases such characters are more elaborate in the male, but occasionally in the female. They are the most astonishing thing that nature has done: brilliant colors, plumes, combs, wattles, and spurs, scent glands (pleasant and unpleasant); red spots, yellow spots, green spots, topknots and tails, horns, lanterns for the dark, songs, howlings, dances and tourneys—a medley of odds and ends" (p. 26).

If we are to discard Hunter's classification, because it is found difficult to determine into which class some of the characters rightly belong, we should have to be dissatisfied with many classifications that are thoroughly well established.

If we limit the term "primary sexual characters" to the reproductive glands, it offers an escape from the difficulties in classifying the prehension organs, as Darwin has pointed out; but it would seem that greater difficulties are met by refusing to place the intromittent organ in the same group with the reproductive glands; and placing it in the group with characters so far removed from "direct connection with the act of reproduction" as, for example, Morgan's list of secondary sexual characters, "brilliant colors, plumes, combs, wattles, and spurs, scent glands (pleasant and unpleasant); red spots, yellow spots, green spots, topknots and tails, horns, lanterns for the dark, songs, howlings, dances and tourneys—a medley of odds and ends." The intromittent organ is not only "directly connected with the act of reproduction"; but it is as much a part of the sex of the individual as the reproductive glands themselves. Any one of the characters in Morgan's entire list of male secondary sexual characters could appear in the female without changing her sex; but the intromittent organ is as clearly indicative of the sex as are the reproductive glands themselves.

If a definite chromosome carries the factors for determining sex and it therefore carries the factors for the reproductive glands, it would seem logical to suppose that the chromosome carrying the factors necessary for the development of the male reproductive glands would also carry the factors necessary for the

development of the intromittent organ which, when present, is functionally a necessary adjunct of the glands, and as indicative of the sex as the reproductive glands themselves. If we cannot accept the mode of transmission of the intromittent organ as an index of the mode of transmission of the reproductive glands, it would seem necessary to discard all structural features or other characters, which are distinctive of the gonads of a given species, such as their distinctions in size, form, color, etc., and assume that these characters, associated with the gland, have a different mode of transmission from the gland itself.

This would prevent any experimental test being applied to the chromosome theories of sex-determination and leave free scope for the wildest cytological speculations. If we should place the intromittent organ in the group of secondary sexual characters, because it has certain features in common with these characters we ought logically to place the reproductive glands themselves in the same group. For example, both these organs, in common with most of the secondary sexual characters, can be transmitted to the opposite sex—hermaphrodites appearing in forms that are normally sexually distinct. A case in point is Goodrichs's '12 interesting and important discovery of a male amphioxus in which 49 of the gonads were testes containing ripe spermatozoa and one was an ovary containing ripe ova. It may be urged that the intromittent organ is a secondary sexual character on the evidence that in the development of the embryo it appears much later than do the gonads—this indicating that the gonads are more fundamental and stable morphological entities. But there are facts opposed to this interpretation—Smith '10 found that when the spider crab is infected by the parasite sacculina, the testes can become so greatly metamorphosed that some of the cells may develop into ova and *the same testis* contain *both* ripe ova and spermatozoa.

It would seem that the division between primary and secondary sexual characters in common with almost all attempts at classification, has the objection that the line of demarcation is not, at all points, perfectly clear; but we believe, in spite of this, that we are justified in classing the intromittent organ as a primary sexual character and that the results from the study of the trans-

mission of this organ may justly be claimed as an index of the method of transmission of the reproductive glands themselves.

Before giving these results we would express our great indebtedness to Professor Poulton and to Dr. Eltringham, of Oxford. We are indebted to Professor Poulton for his kind response to our wish to find an experienced entomologist in England who would be willing to study *E. variolarius* and *E. servus* with the aim of finding other characters than the genital spot that could be studied in the hybrids. He kindly suggested Dr. Eltringham, of Oxford, to whom we are indebted for the discovery that there is a marked specific difference in the intromittent organ of *E. variolarius* and *E. servus*. This discovery has made it possible for us to secure the results which are recorded in this paper.

## Results and Discussion.

The intromittent organs of *E. variolarius* and *E. servus* differ markedly in their length. We have dissected these organs from the genital segment of many of the parent species, and from all the hybrids, both of the $F_1$ and $F_2$ generations. These have been mounted and photographed at a magnification of 20 diameters, and all have been carefully measured at this magnification. The intromittent organ of *E. variolarius* varies in length between 85·5 and 106 mm., while that of *E. servus* varies between 146 and 182 mm. These measurements were made from 62 pure *variolarius* specimens, and from 62 pure *servus* specimens, the mean length of the intromittent organ of *variolarius* being 96·5 mm., and of *servus* 166·41 mm.[1]

Photos 1 to 4 show four typical intromittent organs of *E. variolarius*, these four varying in length between 94 mm. and 98·5 mm. Photos 5 to 8 show four typical intromittent organs from *E. servus*, these four varying in length between 158 mm. and 182 mm. Photos 9 to 12 show four typical intromittent organs of the $F_1$ generation derived from *E. variolarius* ♀ × *E. servus* ♂. These four vary in length from 122 to 132 mm. We have ten intromittent organs of this $F_1$ generation, nine of these being variable intermediates, and one like pure *variolarius*.

[1] A discussion of the mean lengths of the hybrids and of the back-cross, and their bearing on the Mendelian type of inheritance, will be given in a later and more detailed report of these results.

Thus the type of intromittent organ characteristic of the species is transmitted through the female to her male offspring, and also directly by the male, and we may add that this is further proved by the back cross ($F_1$ ♀ × pure *variolarius* ♂). Thus the mode of transmission of this second exclusively male character is like that of the genital spot—*both* of these exclusively male characters being transmitted through the female as well as directly from the male—neither of these characters therefore being sex-linked.

Photos 13 to 20 show eight typical intromittent organs from the $F_2$ generation, these eight varying in length between 85·5 mm. and 140 mm. A few of these specimens (photos 13–15) show that the factors which determine the genital spot and those which determine the intromittent organ are not linked in inheritance (see below).

In our study of the transmission of the genital spot of *variolarius* (Foot and Strobell, '14*a*) we divided the hybrids into three groups—those having a genital spot like that of pure *variolarius*, those without a spot like *servus*, and those with a spot intermediate between these two extremes. In order to compare the results from the study of the two exclusively male characters—the genital spot and the intromittent organ—we have again grouped the hybrids into three classes, those having a length of intromittent organ like that of *variolarius*, those with a length of organ like that of *servus*, and those with a length intermediate between these two extremes.

By this grouping it is possible to compare the genital spot of each individual hybrid with the type of intromittent organ of the same hybrid, in order to determine whether these two exclusively male characters are linked in inheritance—to determine to what extent the two are associated in their transmission. Before discussing this point we shall summarize the points of agreement in the inheritance of the two characters.

The intromittent organ—like the genital spot—is not sex-linked, this being shown by the facts that it is transmitted through the female, and also directly from the male to his male offspring. The intromittent organ—like the genital spot—is transmitted without the aid of either of the so-called "sex-chromosomes." It is transmitted without the aid of the Y-

chromosome because it is inherited through the female, and it is transmitted without the aid of the X-chromosome because it is transmitted *directly* from the male to his male offspring. Like the genital spot, the intromittent organ fails to show dominance in the $F_1$ generation, and fails to show a simple Mendelian ratio in the $F_2$ generation; but the details demonstrating these facts must be reserved for our full report of this work, in which it will be possible to compare the inheritance of these two exclusively male characters in every individual of the $F_1$ and $F_2$ generations.

As in the case of the genital spot, we are forced to conclude that if the factors determining the inheritance of the intromittent organ are carried by definite chromosomes, they must be in *at least* a pair of diploid chromosomes, and as in the case of the genital spot, we are further forced to conclude that there are factors in the cell, outside the chromosomes, which determine just how many of the factors determining the character of intromittent organ shall find expression in the first and second generations of hybrids. The facts show, as in the case of the genital spot, that this cannot be accomplished by the assumed mechanism of division of the chromosomes, but is dependent upon hypothetical factors outside the chromosomes, and thus the distribution of unit factors through the mechanism of chromosome division seems to be an unnecessary assumption. If the factors essential to produce these two exclusively male characters are confined to one chromosome they can be in the Y-chromosome *alone*, for according to the mechanism of the two maturation divisions this is the *only* chromosome that can be in *all* the so-called male-producing spermatozoa. The facts, however, demonstrate that not only the genital spot, but the intromittent organ, can be transmitted without the aid of the Y-chromosome. If, as the facts demand, the factors for these two exclusively male characters cannot be in less than a pair of chromosomes, there seems no adequate reason for confining them to a single pair, or even to the chromosomes at all, for if factors outside the chromosomes and outside the mechanism of the division of the chromosomes, are responsible for the exact expression or total suppression of these characters, this deprives the chromosomes of a most important function which has been attributed to them, based on the mechanism of their division.

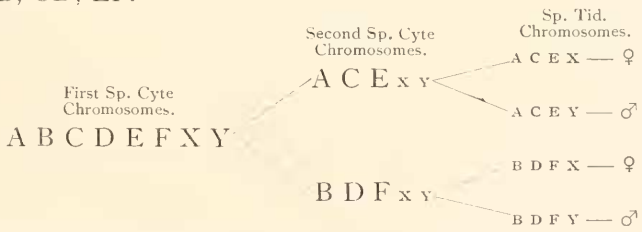
The results from the back cross demonstrate that the type of intromittent organ distinctive of the species can (like the presence or absence of the genital spot) be transmitted by *both* the so-called male-producing and female-producing spermatozoa. The back cross demonstrates not only that the male can directly transmit the intromittent organ distinctive of *variolarius;* but that the type of intromittent organ distinctive of *servus* is transmitted by the $F_1$ female, and therefore was transmitted by the female-producing spermatozoön of the first cross. If such a primary sexual character of the male can be carried by the female-producing spermatozoön, it is only logical to believe that female primary sexual characters also can be transmitted by both types of spermatozoa (male-producing as well as female-producing)—for it is difficult to believe that male and female primary sexual characters differ fundamentally in their method of transmission. The facts appear to deprive the male-producing spermatozoön of its distinctive function, and challenge the logic of endowing slight morphological differences in structures of the cell with causal attributes of fundamental importance.

*Linkage in Inheritance.*—If factors which stand for a given character are carried by a definite chromosome or pair of chromosomes, and the inheritance of the character is due to a special distribution of the factors at mitosis, it would seem logical to expect that the factors of two characters showing a very special mode of distribution (*i. e.*, exclusively male characters) would be contained in the same chromosome, and that this would be indicated by their being linked in the hybrids. We would expect the absence or presence of the genital spot, distinctive of one species, to be associated in inheritance with the type of intromittent organ characteristic of the same species. Even if the extent to which a character appears is dependent upon hypothetical factors outside the chromosomes, we would expect these hypothetical factors to act equally on two characters which are so closely associated as to be contained in the same chromosome. We should expect the two characters never to be so entirely dissociated that we find, in the same individual, the absence of spot characteristic of one species, associated with the type of intromittent organ distinctive of the other species. Instances of

such complete dissociation do however occur and are shown, for example, in photos 13 and 14, which have the length of intromittent organ characteristic of *E. variolarius* (85·5 mm. and 99 mm.) while the specimens from which these organs were dissected have the *E. servus* absence of spot. Photo 15 has a length of intromittent organ almost equal to *E. servus* (140 mm.), while the specimen from which this was dissected has the genital spot distinctive of *E. variolarius*. There are, however, instances of association in the inheritance of the two characters, the intromittent organ and genital spot typical of one of the species occurring in the same $F_2$ individual; but exact classification of the full results shows that the two characters are transmitted quite independently of each other. The intermediates, having a large range of variation, make it possible for many of them to appear to show the two characters in the association that would be in harmony with the chromosome hypothesis, but an exact comparison shows that two plus and two minus intermediates are quite as frequently associated as are a plus and a minus intermediate. If we find such independence in the transmission of the two characters, there seems no logical reason for assuming that their factors are carried by the same chromosome. If their frequent independence in transmission forces us to locate them in at least two of the seven chromosomes contributed by each parent, there seems no adequate reason for confining them to the chromosomes at all, especially as their final mode of expression is not dependent upon the distribution at mitosis of unit factors carried by the chromosomes, but upon hypothetical factors outside the chromosomes.

As the advocates of the sex-determination theory may dismiss these results—as in the case of the genital spot—on the ground that the intromittent organ is not sex-linked, and is merely a secondary sexual character, it may be profitable to attempt to follow theoretically the transmission of what must be admitted are *primary sexual* organs, (the ovaries and the testes), while assuming that they are "sex-linked." This has its difficulties in the case of the testes, for the factors determining the sex-linked characters of authors are assumed to be in the chromosome which is homozygous in one sex and heterozygous in the other

sex. This would place sex-linked factors for the testes in the X-chromosome of these insects; and locating these factors in this chromosome would involve their being in the female-producing spermatozoa only, and this type of spermatozoa therefore would carry the determining factors for *both* the ovaries and the testes, while the male-producing spermatozoa would carry neither the one nor the other. We shall try to show by an analysis of the chromosomes that it is as impossible to associate the testes with the sex-chromosomes as we have shown by experiment is true for the two other exclusively male characters—the genital spot and the intromittent organ. In order to simplify the discussion, we reproduce the following diagram used in an earlier paper to show the method of division of the 14 somatic chromosomes which are distinctive of both *E. variolarius* and *E. servus*. In the diagram we have used the method of designating univalents by the letters of the alphabet, bivalents being represented by AB, CD, EF.




Scheme of the two maturation divisions of *Euschistus variolarius* and *Euschistus servus* based on the assumption that the first maturation division separates autosomes of maternal and paternal origin and the second division halves them. The *XY*-chromosomes on the contrary being halved in the first division and separated in the second division. The relative positions of the autosomes may be changed unless definite chromosomes are always destined to the same pole, but reversing their position in this regard does not alter the end result—that the only chromosome common to both so-called male-producing spermatids is the *Y*-chromosome.[1]

The above diagram demonstrates the two types of spermatozoa, those having the X-chromosome—so-called female-producing; and those having the Y-chromosome—so-called male-producing. If we accept Morgan's conclusion that factors determining all sex-linked characters are located in the X-chromosomes and we assume that the primary sexual characters are sex-linked, this involves placing the factors determining both the ovaries and the

[1] Only six of the twelve autosomes are designated.

testes in the X-chromosomes and, as stated above, this would effectually deprive the so-called male-producing spermatozoa of an essential male-producing function. This is so evidently out of harmony with the chromosome sex-determination theory, that it needs no further comment.

If we attempt to place the factors determining the testes in the other sex-chromosome (the Y-chromosome) we meet difficulties that are equally obvious, for there are many forms that have no Y-chromosome at all. If we could ignore this important fact, we would have, in these insects, quite a diagrammatic demonstration of the chromosome sex-determination theory, for the Y-chromosome is the *only* chromosome that is in *all* the male-producing spermatozoa, just as the X-chromosome is the *only* chromosome that is in *all* the female-producing spermatozoa. Each is the *only* chromosome which is distinctive of the type of spermatozoa which it identifies. But the fact cannot be ignored that the Y-chromosome, so conspicuous in these insects, is absent in most forms, and we must therefore dismiss the possibility that the factors determining the testes of these insects are carried by this chromosome. The association therefore between the testes and the sex-chromosomes can be no closer than we have shown by experiment to be the case between the sex chromosomes and the other two exclusively male characters—the genital spot and the intromittent organ.

Realizing that the Y-chromosome cannot logically function as the carrier of the factors determining a male, Morgan '11 suggested that "the factors for producing the male must be in some other chromosome" (than the Y- or the X-chromosomes). We would consider this suggestion in relation to the factors determining the testes of these insects, ignoring for the present the fact that in his diagram illustrating this suggestion, Morgan does not place these factors in *one* chromosome but in a pair of chromosomes. If we attempt to place the factors for the testes in one of the autosomes, we meet difficulties that are quite as obvious as the difficulties in attempting to place the factors in the sex-chromosomes.

The above diagram (text Fig. 1) shows that the spermatozoa can be classed not only into two types (the so-called male-produc-

ing and female-producing), but each of these groups can again be separated into two types, in relation to their autosome content —one type containing the autosomes A, C, E, and the other type the autosomes B, D, F. As we are discussing the transmission of the testes, the factors for which are presumably carried by the male-producing spermatozoa, we shall consider the two types of these spermatozoa only—those with A, C, E, Y, and those with B, D, F, Y.

Our problem, as stated above, is to determine whether it is possible to place the factors which determine the testes of these insects in one of the autosomes.

A glance at text Fig. 1 shows that each of the autosomes is in *only half* the male-producing spermatozoa, and is also in half the female-producing spermatozoa. If, for example, we assume that autosome A carries the factors for determining the testes, and the X-chromosome carries the factors for determining the ovaries, we shall have *all* the female-producing spermatozoa carrying the factors for determining the ovaries, and in addition to this, half of these spermatozoa will carry the factors for determining the testes, as half of them have the A autosome. The male-producing spermatozoa, on the contrary, will not only carry none of the factors for determining the ovaries, but only half of them can carry the factors for determining the testes, as only half of them have the A autosome.

These conclusions, forced by an analysis of the chromosomes, are by no means in harmony with the demands of the chromosome sex-determination theory—thus it is quite as impossible to confine the factors for the testes to a single autosome, as we have shown is the case with the other two exculsively male characters —the genital spot and the intromittent organ. We might avoid this difficulty by assuming that the maturation divisions of one pair of the autosomes is like that of the XY-chromosomes, and that the factors for the testes are carried by one member of this pair. This involves, however, the further assumption that this autosome must follow the lead of the Y-chromosome or it might arrive in the female-producing spermatozoön. Unless we are willing to make some such unwarranted assumptions, it does not seem possible to make the association between the testes and

the sex-chromosomes any closer than we have shown by experiment is the case between these chromosomes and the genital spot and intromittent organ. Those who would place these factors in the chromosomes must concede that they must be in *at least* a pair of autosomes; there seems indeed no reason for assuming a different mode of transmission for the testes than for the other exclusively male characters—the genital spot and the intromittent organ. We feel we are therefore justified in our claim that the mode of transmission of the genital spot and intromittent organ is an index of the mode of transmission of the reproductive glands themselves, and that our cross-breeding experiments offer direct evidence against the chromosome theory of sex-determination.

If we reconsider Morgan's suggestion that "the factors for producing a male must be located in some other chromosome" than the X-chromosome, and we interpret "some other chromosome" as a pair of autosomes (as Morgan does in his formula) this would locate the factors for the testes in this pair of autosomes and be quite in harmony with our conclusions, that factors for exclusively male organs, if carried by chromosomes, cannot be in less than a pair of chromosomes—and it supports our claim that the method of transmission of the genital spot and intromittent organ is an index of the method of transmission of the testes.

Morgan's formula

Gametes of female—X M—X M
Gametes of male—X M—M.

gives the female zygotes (X M + X M) just as many factors for "producing a male" (M M) as it gives for producing a female (X X) but he does not tell us what determines that the X gametes shall predominate.

According to Morgan's formula the term "female-producing spermatozoön" (X M) would appear to be a misnomer, for it carries "the factors for producing a male" as well as the factors for producing a female. The male-producing spermatozoön, on the contrary, carries the factors for producing a male only (M). The female-producing spermatozoön therefore can transmit exclusively male characters, as we have shown is the case, but

the male-producing spermatozoa cannot transmit exclusively female characters. That exclusively female characters and exclusively male characters should have such a different mode of transmission does not appear to us to be a logical conclusion, but it is a question that it is possible to put to the test of experiment.

The point of view of the investigator as to the chromosome theory of sex-determination seems to be entirely dependent upon the extent of his belief in the individuality of the chromosomes. In these insects, for example, the so-called male-producing spermatozoa have the Y-chromosome and not the X-chromosome, and those who believe in such a degree of individuality of the chromosomes as is demanded by the chromosome hypothesis of sex-determination, must hold that a so-called male-producing spermatozoön *must* develop into a pronucleus with a Y-chromosome and never an X-chromosome. They must hold that the Y- and X-chromosomes are as individual as the king and queen of chess for example.

On the other hand, the cytologist who believes that the chromosomes, like other organs in the cell, are the expression rather than the cause of cell activities, can also believe that there are forces outside the chromosomes that determine whether an egg shall develop into a male or female and can further believe that these forces, acting on the developing pronucleus can cause its chromatin content to develop into the chromosome configuration which is demanded by the sex. As it is impossible to follow the metamorphosis of a spermatozoön into a pronucleus, the cytological proof can probably never be achieved; but there is definite evidence that cells which normally produce certain organs, can be forced by experimental manipulation to produce other organs which have quite different functions, and such a change of function must create a corresponding change of structure, not only in the visible final result, but in the initial changes of the cell itself. Thus we believe that the structure of the cell, or any part of the cell is not the determining factor, but is merely an expression of other forces.

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## Description of Plate I.

The length of the intromittent organs was measured by a small pair of architect's dividers fitted with No. 9 needle points, and the dividers were frequently tested by measuring a 100 mm. line. Each intromittent organ was photographed at a magnification of 20 diameters, and the measuring was simplified by numbering each division of 20 mm. by a pencil mark. The measurements were taken from the distal end of the intromittent organ at the point where the coil enters the gland, the coil being easily dissected off at this point (photos 3, 4, etc.).

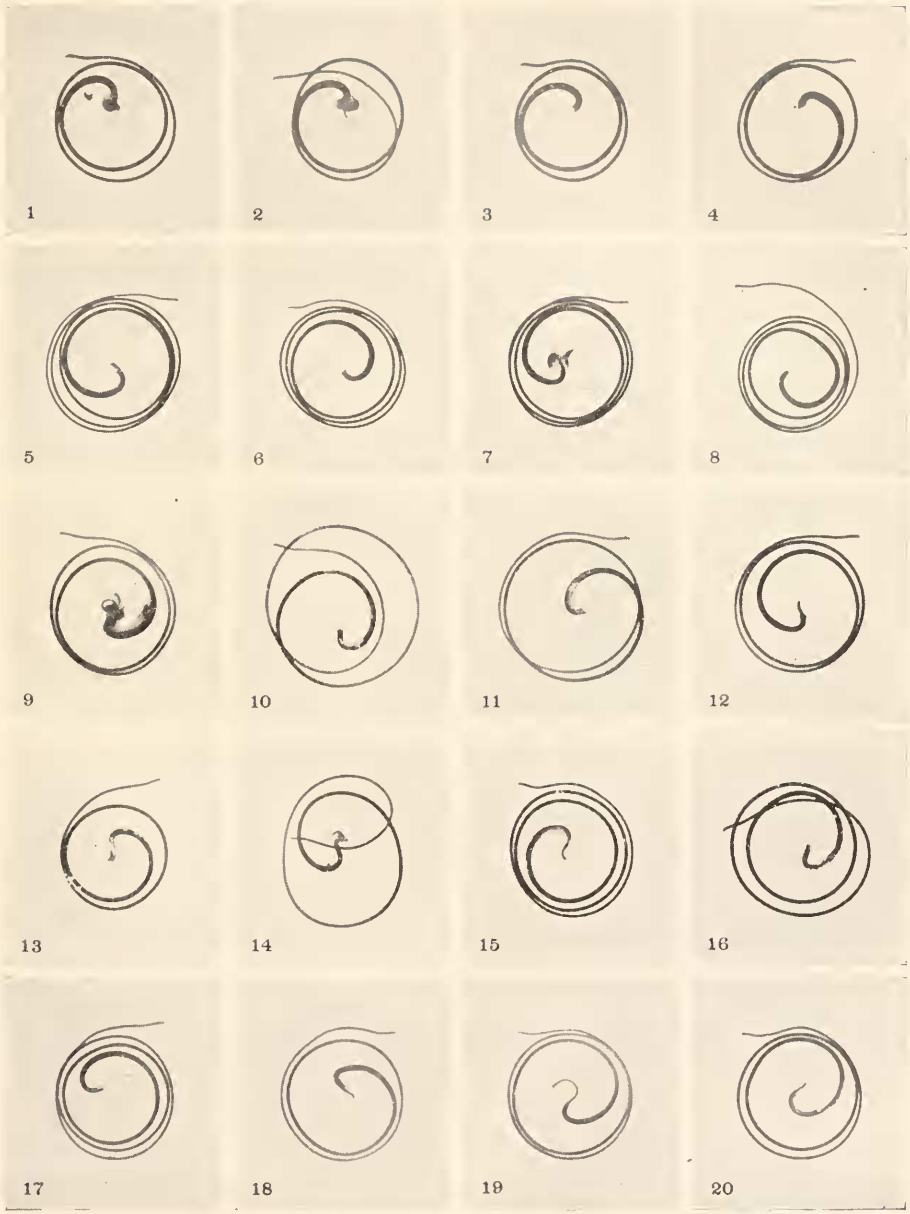
In photos 1, 2, 7, 9, a small part of the gland itself is retained. When part of the intromittent organ that is within the gland is preserved, the point from which the measurement was taken is easily determined, for the part within the gland is transparent and tapers at this point to a much smaller canal, *e. g.*, photos 15 and 19.

Photos 1 to 4. Typical specimens of the intromittent organ of *Euschistus variolarius*. Length of the organ of photo 1, 98·5 mm. Photo 2, length 97·5 mm. Photo 3, length 95 mm. Photo 4, length 94 mm.

Photos 5 to 8. Typical specimens of the intromittent organ of *E. servus*. Length of the organ of photo 5, 182 mm. Photo 6, length 158 mm. Photo 7, length 170 mm. Photo 8, length 162 mm.

Photos 9 to 12. The intromittent organs from four of the $F_1$ hybrids. Length of the organ of photo 9, 124 mm. Photo 10, length 132 mm. Photo 11, length 122 mm. Photo 12, length 126 mm.

Photos 13 to 20. The intromittent organs from eight of the $F_2$ generation. Length of the organ of photo 13, 85·5 mm. Photo 14, length 97 mm. Photo 15, length 140 mm. Photo 16, length 128 mm. Photo 17, length 147 mm. Photo 18, length 100 mm. Photo 19, length 124 mm. Photo 20, length 124 mm.

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# BIOLOGICAL BULLETIN

## IS THE FERTILIZATION MEMBRANE OF ARBACIA EGGS A PRECIPITATION MEMBRANE?

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In 1912 McClendon ('12) suggested that the fertilization membrane of sea urchin eggs is a precipitation membrane formed when two colloids of opposite electrical charge meet, namely the negative egg jelly (mucous, zona pellucida) and a positive substance secreted by the egg. Elder ('13) has advanced a similar view and recently McClendon ('14) has restated his former opinion. Of course the test of the theory is perfectly simple. An unfertilized egg from which the jelly has been removed should form no membrane when fertilized, and both McClendon and Elder state that this is the case.

I have utterly failed to confirm these statements and find that whether an egg forms a normal membrane or not is absolutely independent of the jelly which surrounds it. My method of determining this is as follows:—Eggs from one female are divided into two parts and the jelly removed from the eggs of one part by shaking two or three times in a test-tube and washing with sea water. Eggs of both lots are placed together upon a slide and india ink suspension, sperm, and, after mixing, a cover glass is added. It is perfectly easy to see which eggs are surrounded by jelly and which are not. All of them, whether with or without jelly, form fertilization membranes which in the two-cell stage surround the whole egg and are quite distinct from the hyaline plasma layer which is close to each blastomere. An egg without jelly touching the jelly of another egg should form a membrane, according to McClendon's idea, only on the side toward the jelly. Yet such a condition is never observed, but instead a membrane forms about the whole egg. Thus eggs without jelly will form fertilization membranes.

If *Arbacia* eggs are allowed to stand for about 52 hours at a temperature of 22° C. or for 3 to 4 days at a temperature of 12° C. no membranes form despite the fact that they may still be surrounded by a copious amount of jelly. This observation so far as I am aware was first made by Loeb ('03) and confirmed by myself ('10) and F. R. Lillie ('14). In the two-cell stage the blastomeres are widely separated because not surrounded by a fertilization membrane although the hyaline plasma layer is clearly visible.[1] Thus even an egg surrounded by jelly may fail to form a fertilization membrane.

I at first thought that McClendon's observations were due to the fact that he took so long a time in removing the jelly, (agitation and washing for "not more than 24 hrs.") that the mere age of the eggs would account for their inability to form membranes. I find, however, that the eggs *with jelly* must stand for over 52 hrs. at 22° C. before they are unable to form a fertilization membrane.

This fact suggests that the membrane-forming substance (membranogen) which passes out of the egg to form the membrane gradually diffuses away or is used up when the egg is allowed to stand. Since the membranogen is probably a protein we should expect it to diffuse away from the eggs without jelly much more readily than from those eggs with jelly. It is well known that colloids do not readily diffuse through each other. Such is actually the case and in this point lies, I believe, the explanation of McClendon's results. If we take the eggs from one female, remove the jelly from one lot by shaking, but allow it to remain on the other lot, both lots will form perfectly normal membranes if fertilized immediately. If both lots are allowed to stand for 24 hrs. and are then fertilized the eggs which have stood without jelly form no membranes while those with jelly form membranes only slightly thinner than normal. Membrane

[1] The hyaline plasma layer appears much thicker than in freshly fertilized eggs and in my previous paper ('10) I described this as a special type of membrane. However there is nothing present at all comparable to the normal fertilization membrane and without quibbling over minute distinctions we may safely class these eggs as "without membranes." McClendon ('14) misquotes me in stating that I believe the fertilization membranes to be present on unfertilized eggs and to be simply lifted off after fertilization. I have held exactly the opposite view (Harvey, '10).

formation is not, then, a question of presence or absence of jelly but is dependent on the time the eggs have stood. We may imagine this to be due to the loss of some membrane-forming material from the eggs which much more readily takes place when the mechanical hindrance of the jelly is removed.[1]

These experiments point against the view that the fertilization membrane is a precipitation membrane.

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[1] According to Kite ('12) membrane formation is merely the swelling of a fine invisible (unless stained) vitelline membrane together with a change of its optical properties. If that is true the inability of eggs which have stood for some time without jelly to form fertilization membranes would seem to be due to a loss, through solution, of the vitelline membrane.

# SO-CALLED PARTHENOGENESIS IN THE WHITE MOUSE.

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In atretic follicles of ovaries in a number of different mammals the oöcytes go through a process which somewhat resembles maturation. Various stages of mitosis are seen and frequently a first polar body is present. In some cases the oöcyte is found divided up in a number of small parts some of which contain nuclei. This process has been described as a beginning of parthenogenetic cleavage and also as degenerative fragmentation. Bonnet ('00) gives a review of the work done up to that time and after considering all the evidence decides that the mitotic figures seen in such egg-cells are not those of parthenogenetic cleavage but are rather those of more or less abnormal maturation stages. Newman ('13) reviews briefly the work done since Bonnet's paper and presents the results of his studies on the armadillo in support of the view that "a limited amount of parthenogenetic cleavage occurs but that development proceeds no farther than two or three cell divisions." Van der Stricht ('01), who worked on the bat claims that in that form is found a beginning of true parthenogenesis. Rubaschkin ('07), who studied the guinea-pig, and Athias ('09), for the dormouse, state that the phenomena are to be interpreted as degenerative fragmentation which at the most merely resembles parthenogenesis.

In material which I have been preparing for a study of oögenesis in the white mouse I found that in the ovaries of young mice approaching sexual maturity there is a very extensive degeneration of follicles, most marked between the ages of twenty-five and forty days. As the work on this problem has all apparently been done on the ovaries of fully or young adult mammals, it was thought worth while to use the material in these immature ovaries[1] for a study of this so-called parthenogenesis.

[1] The material used for this work consists of ovaries of white mice varying in age from twenty to ninety days. These ovaries were fixed in Carnoy's fixer

In the white mouse, it is certain that the changes taking place in the oöcytes are in some way correlated with the atresia of the follicles, for in follicles which have not begun to degenerate the egg-cells are normal in appearance and the nuclei are normal resting nuclei. But in follicles overtaken by atresia the cytoplasm of the oöcytes is found to stain more deeply with acid stains, fat granules are found in large numbers, and the nuclei are in various stages of mitosis. The degeneration of the follicle in some way stimulates the oöcyte so that it passes through more or less abnormal maturation stages.

The early prophase is probably passed through very rapidly as no stages were seen of nuclei between the resting stage and the equatorial plate stage. Lams and Doorme ('08) did not observe the stages between the resting nucleus and the first polar spindle in the normal maturation of the egg of the white mouse. Kirk-

("6:3:1"), Hermann's, and Flemming's fluids. Heidenhain's iron hematoxylin, Jenner's blood stain, and Flemming's triple stain were used. Sections were stained over night in Jenner's stain, diluted with three parts of water; this stain was used after fixation in Carnoy's fluid and gave excellent results for some purposes. The cytoplasm of degenerating egg-cells was stained a much deeper pink or red than that of normal egg-cells. The nuclei of follicle cells and phagocytic cells were stained a deep blue. The stain will fade after a time, however.

A modification of the shorter method of Flemming's triple stain was used. Sections of material fixed for two to four hours in Flemming's or Hermann's fluid were bleached in a dilute solution of hydrogen peroxid and after rinsing were placed in a four per cent. solution of ferric alum for four to twelve hours. The sections were then rinsed in distilled water, dipped in the safranin solution a second or two, rinsed again in distilled water, and placed in the gentian violet solution for two to ten or fifteen minutes. Then after rinsing in distilled water the sections were stained in the orange G for ten to sixty seconds. After dehydrating rapidly in absolute alcohol the sections were differentiated in clove oil under the control of the microscope. The clove oil was removed by toluene or xylene and the sections mounted in balsam. By this method the cytoplasm of the oöcytes was stained a yellow-brown, the chromosomes were stained a violet, and the spindle fibers a dark violet—almost purple. The method is rather capricious but when successful, the spindle fibers stand out very distinctly against the yellow-brown cytoplasm of the egg-cell. The method was used principally to bring out the spindle fibers, as the chromosomes are not stained so distinctly or sharply as by the iron hematoxylin method.

The solutions used are as follows:

1. { Safranin, saturated solution in absolute alcohol, 1 part.
   { Safranin, saturated solution in distilled water, 1 part.
2. Gentian violet, 1 per cent. solution in distilled water.
3. Orange G, 2 per cent. solution in distilled water.

ham ('08) describes very briefly a few stages of the prophase of the first maturation division in this same form.

Descriptions of the first and second polar spindles in normally maturing oöcytes do not agree. Sobotta ('07) says: "Wenn auch namentlich die Breite individuell etwas wechselt, so beträgt Länge wie Breite des ersten Richtungsspindel der Maus doch stets das Doppelte der Masse des zweiten Richtungsspindel, die Breite ist in der Regel mehr als doppelt so gross." Lams and Doorme, on the other hand, find that the first and second polar spindles are of exactly the same length and diameter. They state: "D'une façon certaine, un ovule à second fuseau ne se distingue d'un ovule à premier fuseau que par la présence, dans le premier cas, du premier globule polaire." Kirkham agrees with Sobotta in saying that the first polar spindle is larger than the second and also in describing the chromosomes as differing in size and shape in the two spindles.

In regard to oöcytes in atretic follicles, Athias says of the spindle, first or second: "Sa forme et ses dimensions sont variables, mais il n'y a pas des caractères qui soient propre au premier ou au second fuseau; la présence concomitante d'un premier globule polaire est, d'après ce que j'ai pu constater dans mes préparations, le seul critérium pour affirmer si l'on est en présence d'un second fuseau de direction." In my own preparations the lengths and diameters of a number of first and second polar spindles in atretic oöcytes were measured. For the first polar spindles, the average length was found to be 24.7 $\mu$, the diameter 12.8 $\mu$, while for the second, the average length was 25.9 $\mu$ and the diameter 8.7 $\mu$. Allowing for error in measuring, the spindles are seen to be of about the same length, while the second polar spindles are about two thirds the diameter of the first.

In degenerating oöcytes of the white mouse the first polar spindles are found to be of two general forms: slender, with the achromatic fibers meeting at a point at each end, and thick, "barrel-shaped," with broadly rounded ends. These two kinds are met with in about equal numbers. In both, the chromosomes are arranged with their long axes parallel to that of the spindle. The chromosomes are not arranged around the periphery of the

spindle, but are scattered in the plane through the middle of the spindle, at right angles to its length. The chromosomes of the first polar spindle are larger than those of the second and are long with a marked thickening or swelling at the middle. This thickening is at one side of the chromosomes and it is at this point that division takes place. The division is apparently transverse. This account agrees with those of Sobotta and Kirkham for the normal first maturation spindle.

The chromsomes are grouped so closely together and overlap to such an extent that it is difficult to determine their number with accuracy. They apparently vary in number from twelve to twenty-four, the larger numbers being due to a precocious division of some, while others are still undivided.

Descriptions of the appearance of the spindle itself differ. Sobotta, Kirkham, and Lams and Doorme, state that polar radiations or asters are not present in first polar spindles of normally maturing oöcytes, and Athias agrees with them in the case of degenerating egg-cells. Rubaschkin, however, describes in atretic oöcytes of the guinea-pig polar radiations arising from a clear area or centrosphere. Kirkham describes centrosomes consisting of several minute granules at the poles of the spindles. Sobotta states that there are no centrosomes in the first polar spindles of normally maturing oöcytes, and Athias finds none in degenerating egg-cells. In my own preparations radiating fibers are to be seen at the poles of a few first polar spindles; these, however, are not to be considered true asters, but spindle fibers which have broken away from the spindle. This will be discussed more in detail further on. No centrosomes are found in any of these degenerating oöcytes. The spindle fibers are not divided into central spindle fibers and mantle fibers; the fibers with chromosomes attached are intermingled with those which are not connected with chromosomes. The two kinds do not differ in appearance or staining reaction. The spindle itself usually lies at right angles to the radius of the oöcyte, until it swings around to a radial position for the formation of the polar body. (See Figs. 1, 2 and 5.)

The stages in the formation of the first polar body must follow one another rapidly for only a few of these stages were

observed. Some of the chromosomes divide earlier than others and consequently the metaphase is not so distinctly marked as in some forms. A few instances of a telophase were seen, in some of which both groups of daughter-chromosomes are in the oöcyte, with no indication as yet of a division of the cytoplasm to form the polar body, while in the others the constricting off of the polar body may be plainly seen. After the first polar body is formed the chromosomes remaining in the oöcyte do not form a resting nucleus but at once enter the second polar spindle.

The second polar spindle, as stated above, is of about the same length as the first, while only two thirds or three fourths as much in diameter. The chromosomes as seen in the equatorial plate stage are short and rod-like and straight or slightly curved. They are not as long or as much curved as Kirkham describes in the second polar spindles of normally maturing oöcytes. The chromosomes are arranged with their long axes parallel to that of the spindle itself and as in the first polar spindle are scattered in a plane at right angles to the length of the spindle. This differs from Kirkham's account of the normal second polar spindle, in which he states that in general the chromosomes lie with their long axes across the spindle. In the spindles of these degenerating egg-cells some of the chromosomes are constricted across the middle in preparation for division, having the appearance of elongated dumb-bells; others have not started to divide and have the typical rod-like form. Others still have already divided and are short and thick, their length only slightly exceeding their diameter. (See Figs. 3 and 4.) This division as well as the first is apparently transverse in the mouse. The chromosomes are crowded together, as in the first polar spindle, making an accurate count difficult; there are from twelve to thirty, owing to the fact that some divide sooner than others.

Descriptions of the appearance of the second polar spindle are as conflicting as those of the first. Sobotta states that in normally maturing oöcytes there are neither centrosomes nor polar radiations. Lams and Doorme describe centrosomes but say that polar radiations are absent; Kirkham states that both centrosomes and polar radiations are present in some cases. In second polar spindles of degenerating oöcytes Athias states

that neither centrosomes nor polar radiations are present. In my own preparations radiating fibers are to be seen at the poles of a number of second polar spindles. As in the case of the first polar spindles, however, these are not to be considered true asters, but spindle fibers which have broken away from the spindle, and have assumed a radial position at the ends of the spindles. This will be discussed more in detail later. In general, centrosomes are absent in second polar spindles of atretic oöcytes, but are present in some cases. When they are seen, they consist of a few minute granules which stain deeply and are either in a compact group at the poles of the spindle or are somewhat spread out forming a sort of cap for the spindle. (See Figs. 3, 4 and 6.)

The first polar body is almost always present with the secondary oöcyte in atretic follicles, although in a few instances the spindle has all the characteristics of a second polar spindle while the polar body is not to be seen. In such cases it is possible that the polar body has already degenerated and been absorbed, or as Kirkham suggests for normal oöcytes, it may have been "forced through the zona (pellucida) by the contraction of the latter under the influence of changing osmotic conditions" during fixation. In nearly every instance, however, the polar body is present, lying within the zona pellucida, and somewhat flattened between the oöcyte and the zona. The smaller dimension of the polar body is one half or two thirds of the larger, while the larger diameter itself is a fifth to a third that of the oöcyte. In a few cases the polar body contains a spindle more or less deranged and abnormal (see Fig. 3) but usually the chromosomes are scattered through it irregularly. They may be grouped in a few large irregular masses of chromatin or there may be a number of smaller chromosomes of abnormal size and shape. In a few cases a resting nucleus may be seen in the polar body (Fig. 10). The second polar spindle is usually found in the oöcyte near the polar body, at right angles to the radius of the egg-cell. Rarely it may be seen in the other side of the oöcyte, and a few spindles have been seen in a radial position. A few instances were observed where the oöcyte contained two spindles; this is probably due to the fact that the egg-cell had two nuclei to start with.

Up to this point oöcytes in follicles undergoing follicular atresia have passed through the same stages, with some differences in detail, as normally maturing egg-cells. The later stages however are different. The next step in degeneration is the breaking down of the spindles. The usual course is for the oöcytes to form the second polar spindles which then break down; but if degeneration has proceeded a little more rapidly, this fate may overtake the first polar spindles before the polar body is formed.

As stated above, in the first polar spindle as well as in the second, the achromatic fibers are all intermingled, those with chromosomes attached and those without, and do not differ in appearance or staining reaction. The fibers with no chromosomes attached to them break across at their middle and the free ends move out in the cytoplasm. As the polar ends remain attached to the poles of the spindles, the formation of "asters" is brought about. Stages are seen (Figs. 5 and 6) in which the breaking or splitting off of the fibers is taking place; some of the fibers have just broken across, in the figures, and others have already assumed a radial position, giving the appearance of "asters." The achromatic fibers with chromosomes attached next break or split off, and as their free ends move out into the cytoplasm, they draw after them the attached chromosomes (see Fig. 7). In this way more fibers are added to the "asters" and chromosomes are seen connected with the ends of some of the fibers. This splitting off of the achromatic fibers explains the fact that some spindles have radiating fibers or "asters" at their poles, while in others they are absent. The oöcytes containing spindles without radiating fibers have not advanced so far in degeneration that the spindle fibers have begun to split off. The result of this splitting off of the fibers and the consequent breaking down of the spindles is that the chromosomes are scattered in all directions in the cytoplasm at each pole of the spindle, while still connected with the poles by the spindle fibers.

The cause of the breaking down of the spindles is to be found in the degeneration of the oöcyte. That this degeneration has proceeded to quite an extent is shown by the presence in the cytoplasm of fat-granules and crystalloid bodies, and by the

fact that the cytoplasm stains much more deeply with acid stains such as eosin and orange G than does the cytoplasm of normal oöcytes. The spindle fibers share in this degeneration and show it first by breaking across and splitting off from the spindle. Rubaschkin states that as the fibers split off, the poles of the spindles approach each other and finally come to lie so close together that it is difficult or impossible to distinguish one from the other. While this account of the breaking down of the spindles agrees essentially with that of Rubaschkin for the guinea-pig, nothing resembling the approach of the poles of the spindles was observed in the mouse.

The achromatic fibers soon disappear and the chromosomes thus left free in the cytoplasm of the oöcyte begin to form nuclei. Each chromosome forms a small vesicle which has the appearance of a vacuole with the chromatin material massed at one side (Fig. 13). In some instances the chromatin is arranged in small granules around the outer part of the vesicle (Fig. 8). As this process goes on, the vesicles near enough together coalesce to form larger ones (Figs. 8, 9, 11), while those isolated in the cytoplasm remain separate. In this way a varying number of nuclei are formed, of different sizes. A nucleus formed by the combining of a number of chromosomes is larger than one formed from a single chromosome. The final number of nuclei thus formed may be from two to twelve, depending on how the chromosomes were scattered in the oöcyte. These nuclei are transformed into resting nuclei of more or less normal appearance.

The nucleo-cytoplasmic relationship, already interfered with by the degenerative changes in the egg-cell, is further disturbed by this formation of a number of small nuclei. The size-relationship, as well as the morphological, physiological, and chemical, relationship, is clearly affected. Apparently there is an effort, even in the degenerating oöcyte, to restore as far as possible this size-relationship, and this effort is expressed by a breaking up of the cytoplasm into smaller parts around the various nuclei. A part of the cytoplasm may surround several of these small nuclei when these are close together, or may enclose only one, when they are isolated. It occasionally happens that a bit of the cytoplasm may fail to contain even one of these nuclei, when

part of the egg-cell was without any nuclei as a result of an incomplete scattering of the chromosomes. The result of this breaking up of the oöcyte is that there are formed a number of small "cells," some with several nuclei, some with one, and some with none, so that the oöcyte has the appearance of a "morula." The fact that some "cells" have nuclei and others have not, is due to the uneven distribution of the chromosomes in the cytoplasm of the oöcyte when the spindle breaks down. In general, the "cells" containing large nuclei, or a group of nuclei, are larger than those with one nucleus or none. However, a definite or effective control over this fragmentation is apparently lacking.

Several authors, Newman among others, have described cells in this "morula" stage which have spindles in them, and state that these are cleavage spindles and that therefore this is a case of parthenogenetic cleavage. It is more probable, however that in such cases the cell containing the spindle is the first polar body, which, as noted above, occasionally forms a spindle, and which may in rare instances divide. In the white mouse no spindle was found in any of the cells of this "morula" stage.

In the cells of the "morula," and sometimes in the oöcyte before it has fragmented, are frequently found crystalloid bodies the nature and origin of which are unknown. Possibly they are a product of the degenerative changes in the egg-cell. Fat granules are found in the oöcytes in increasing numbers as degeneration goes on.

In a few instances the oöcyte is found to have formed two cells of nearly equal size, each containing a nucleus. Van der Stricht describes such cases in the bat and states that each cell may divide again, and each of the four cells thus formed may also divide. The formation of two such equal cells may be explained on the grounds that the scattered chromosomes were arranged in two groups and formed two nuclei; the oöcyte then broke up into two fragments of equal size. Such an egg-cell is shown in Fig. 10, with the first polar body also present; but the two nuclei are not equal in size, nor normal in appearance. In fact, one is apparently little more than a vacuole.

The fate of the "morula" may be briefly described. The zona pellucida usually persists as a thick transparent membrane

for some time after the oöcyte itself has completely degenerated and disappeared, although in a few instances it is absorbed early. Phagocytic (?) cells make their way into the oöcyte through the zona pellucida, and are probably to be regarded as follicle cells from the degenerating follicle. These cells are usually seen in the outer border of the oöcyte, or just outside it, lining the inner surface of the zona pellucida, sometimes as early in the course of degeneration as the spindle stage. There are not many of these cells in a single oöcyte, not more than eight or ten and frequently no more than three or four. They may be imbedded in the cytoplasm of the egg-cell and in the "morula" stage are frequently seen in between the separate cells. One case is illustrated (Fig. 14) showing one of these extra-ovular cells just after it has entered the oöcyte, still retaining its connection with other follicle cells outside the egg-cell by means of a protoplasmic process extending through the zona pellucida. This same cell is also shown to be connected with one or two other similar cells within the zona by other protoplasmic processes, forming a sort of syncytial net-work or mesh-work in among the fragments of the degenerating egg-cell.

The cytoplasm of these cells is usually rather scanty and sometimes they look like bare nuclei imbedded in the cytoplasm of the oöcyte (Figs. 8 and 9). They are not, however, to be confused with the nuclei of the oöcyte formed by the breaking down of the spindle, for they react differently to the stains used and have a different structure. They are finely granular and these granules are stained an intense black by iron hematoxylin and deep blue by Jenner's stain.

It may be through the action of these cells that the fragments of the oöcyte are gradually absorbed and disappear, for later on the zona pellucida is seen, shrunken and distorted, with a few of these cells in a remnant of the egg-cell. In still later stages, these cells are seen alone inside the zona, and this condition may persist for some time (Fig. 16). Eventually the zona pellucida and these cells all disappear and by this time the follicle itself has usually completely degenerated.

Thus it is seen that the oöcytes in atretic follicles in the ovary of white mice not yet sexually mature undergo a series of changes

which in the early stages at least resemble maturation. That these changes are in some way correlated with the atresia is shown by the fact that all the oöcytes exhibiting these phenomena are found in atretic follicles, and egg-cells of normal appearance are seen in the follicles not yet overtaken by atresia. The degeneration of the follicle stimulates the oöcyte to pass through a process which at first resembles maturation but which later results in a breaking up of the egg-cell into fragments, some with nuclei and some without. In the light of the evidence here presented, this process can not be considered parthenogenetic cleavage for no mitotic figures other than those of a more or less abnormal maturation were seen; and if this were true parthenogenetic cleavage it would be expected that some stages of mitosis would be observed. The absence of mitotic figures other than more or less normal polar spindles, the breaking down of these spindles, the scattering of the chromosomes, the formation of nuclei from these chromosomes, and the consequent breaking up of the egg-cell into small parts, with or without nuclei, show rather conclusively that in the white mouse, not yet sexually mature, the process is one of degenerative fragmentation.

## Summary.

The spindles seen in oöcytes in follicles undergoing atresia folliculi are maturation spindles, more or less abnormal, and not cleavage spindles.

By the splitting off of the achromatic fibers and the consequent breaking down of these spindles the chromosomes are scattered through the cytoplasm of the oöcyte and form a number of nuclei.

The nucleo-cytoplasmic relationship, disturbed by the degenerative changes in follicle and egg-cell, causes the oöcyte to break up into fragments, some with one or more nuclei and some with none. These fragments are gradually absorbed, probably through the action of phagocytic cells of follicular origin, and disappear.

The process is one of degenerative fragmentation and not parthenogenetic cleavage.

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## EXPLANATION OF PLATES.

All the figures are camera-lucida drawings made from the actual preparations. All the drawings except Figs. 3, 7, and 14, were made by Miss Cora J. Whitman.

### PLATE I.

FIG. 1. Primary öocyte, containing a first polar spindle. The chromosomes are longer and more slender than usual in first polar spindles. The zona pelludica is seen surrounding the egg-cell (zp.). × 670.

FIG. 2. Primary oöcyte containing a first polar spindle, of the "barrel-shaped" type. The chromosomes are of the type usual for this spindle. The zona pellucida has disappeared. × 670.

FIG. 3. Secondary oöcyte, with second polar spindle and polar body which also contains a spindle more or less deranged. Some of the chromosomes of the egg spindle have been omitted from the drawing in order to show more clearly the characteristic shape of the chromosomes of the second polar spindle. × 670.

FIG. 4. Secondary oöcyte, with second polar spindle and polar body. Centrosomes are seen at each end of the spindle. × 916.

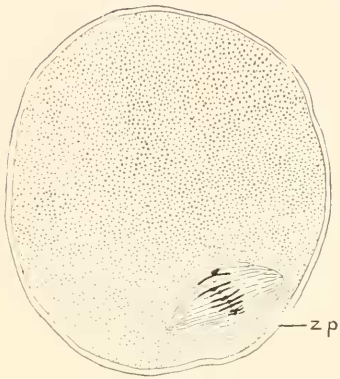



Fig 1

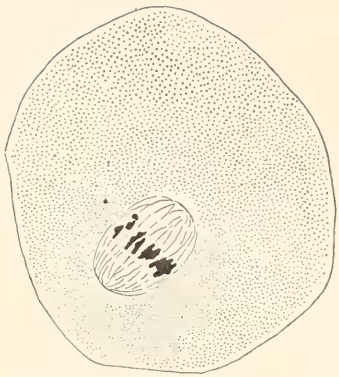


Fig 2

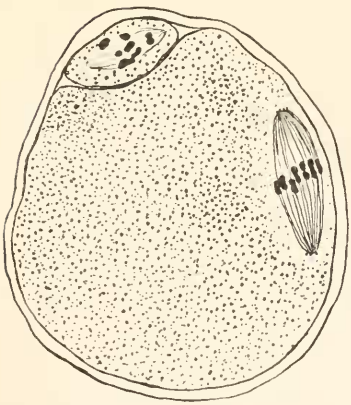


Fig 3

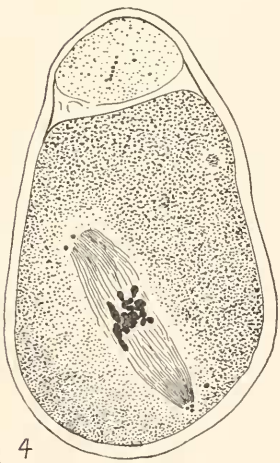


Fig 4

H. M. KINGERY.

PLATE II.

FIG. 5. First polar spindle alone, showing achromatic fibers splitting off, forming "asters." × 916.

FIG. 6. Second polar spindle alone, showing well-defined "asters," formed by fibers which have split off from the spindle. × 916.

FIG. 7. Primary oöcyte showing spindle seen obliquely from one end, which has broken down. The scattering of the chromosomes is partially accomplished. ×670.

FIG. 8. Egg-cell with nuclei formed from scattered chromosomes. The bodies at the end of the oöcyte (*x*) are probably cells formed by the division of the polar body. A crystalloid body (*c*) and an extra-ovular cell (*p*) are also shown. ×670.

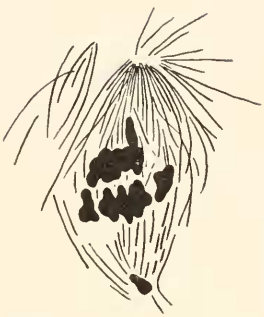


Fig 5

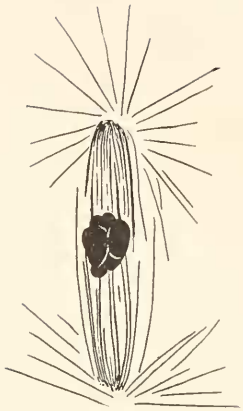


Fig 6

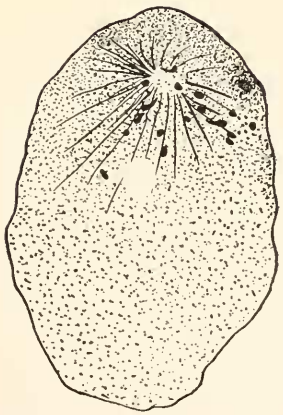


Fig. 7.

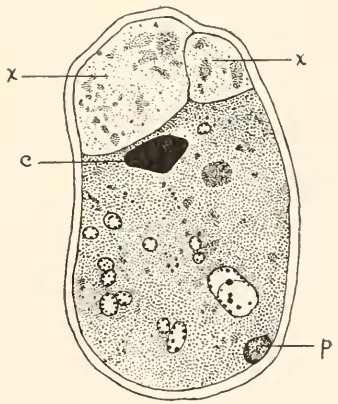



Fig 8.

PLATE III.

FIG. 9. Egg-cell containing four nuclei; these, from their size, have been formed by the coalescence of a number of smaller vesicles. Two extra-ovular cells (*p*) are shown. × 670.

FIG. 10. Oöcyte divided into two more or less equal parts, with polar body also present (*pb*). The nuclei are not alike, one being apparently only a hollow vesicle. × 670.

FIG. 11. Egg-cell in several fragments, one of which contains five nuclei. The zona pellucida is broken in two places and extra-ovular cells are present between the fragments. × 670.

FIG. 12. "Morula" stage, some fragments with nuclei and others without. × 670.

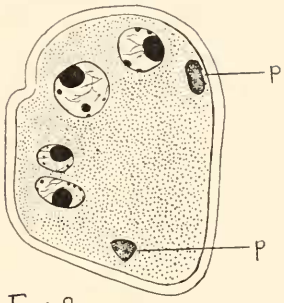



Fig 9

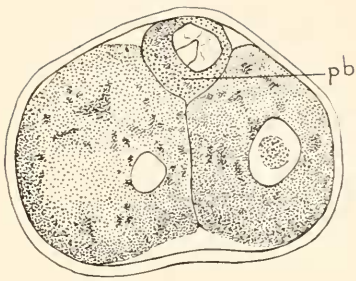



Fig 10

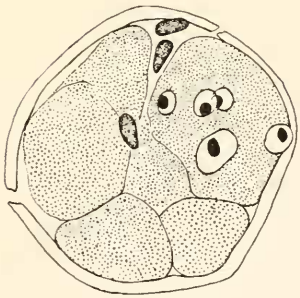


Fig 11

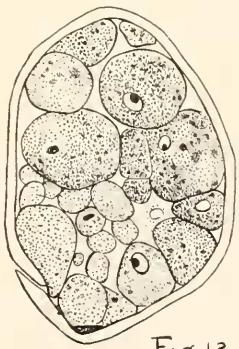


Fig 12

PLATE IV.

FIG. 13. Egg-cell showing small nuclei formed from the scattered chromosomes. The chromatin material is massed at one side of each of the vesicles. × 670.

FIG. 14. Egg-cell containing extra-ovular cells connected by protoplasmic processes. One is still connected with the follicle cells outside by a process extending through the zona pellucida. × 670.

FIGS. 15 AND 16. Final stages in degeneration. Zona pelludica with remnant of oöcyte and a few extra-ovular cells inside. × 670.

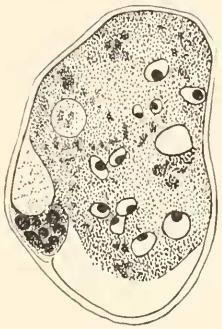


Fig. 13

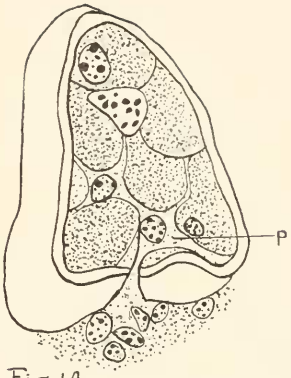



Fig 14

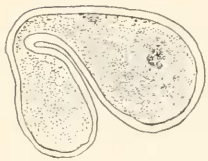


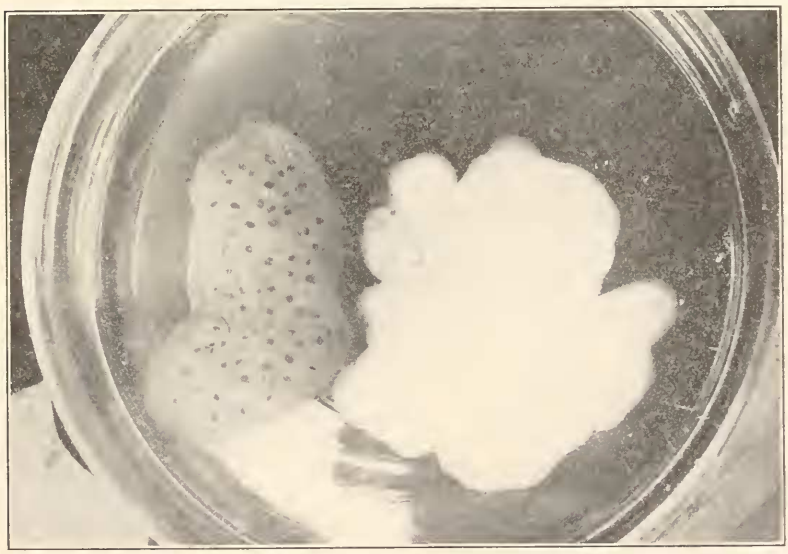
Fig 15

Fig 16

# A MILKY WHITE AMPHIBIAN EGG JELL.

ARTHUR M. BANTA AND ROSS AIKEN GORTNER.

While collecting *Ambystoma punctatum* eggs in April, 1914, the writers found a freshly-laid bunch which had jell of milky whiteness instead of being transparent. The white clutch of eggs was conspicuous among the others even though the jell had as yet swelled very little and the bunch was quite small. The eggs were still undivided. They had the normal amount of pigment. The jell was so clouded that at first no eggs could be seen in the mass without breaking into the jell. The jell imbibed water and the egg bunch soon assumed the normal proportions for the



clutch of this species. The photograph shows the relative opacity of the white bunch and a normal bunch of eggs of the same age. Only a few eggs were visible in the opaque bunch when the photograph was taken while one could readily see the bottom of the dish through the jell of the normal bunch. The individual outer and inner egg membranes in the white bunch were of normal transparency. This is indicated indistinctly

in the photograph by the somewhat more transparent spots, in which were eggs, near the edges of the white bunch.

When just ready to hatch the embryos with their egg membranes were removed from the jell, care being taken to remove all traces of eggs which had died during development. The water was carefully drained off and the jell was dried in a porcelain dish over a water bath. The jell from a normal egg bunch of the same stage of development was treated in a similar manner. After drying on the water bath the jell was further desiccated in a vacuum desiccator over sulfuric acid.

233 grams of normal egg jell yielded 0.7855 grams or 0.337 per cent. of dry material.

294 grams of the opaque jell yielded 1.060 grams or 0.361 per cent. of dry material.

It thus appears that the jell which normally surrounds these eggs contains about 99.6 per cent. of water and yet this jell is fairly tough and resistant and admirably suited to supporting the eggs in such a position as to permit ready diffusion of oxygen and carbon dioxide and to protect the eggs from mechanical injury.

The two jells when desiccated were indistinguishable. A portion of each was again placed in water and in a short time they had imbibed enough water to resume their former appearance, the one becoming milky white and the other transparent. It may perhaps be germane to add that the milky appearance could not be ascribed to bacteria inasmuch as the egg mass was found shortly after being deposited and also because there was no evidence of bacterial decomposition up to the time the drying was begun.

Thinking that perhaps the milky color was produced by an admixture of albumen with the mucin (which composes the normal egg jell) nitrogen determinations were made of both abnormal and normal egg jells.

The nitrogen was determined by Kjeldahl's method.

*Normal egg jell.*—0.2060 gram gave 12.3 c.c. 0.1 normal $NH_4OH$; 0.3564 gram gave 21.1 c.c. 0.1 normal $NH_4OH$, indicating 8.36 per cent. and 8.29 per cent. respectively or an average of 8.32 per cent. of nitrogen in the normal egg jell.

*Opaque egg jell.*—0.2764 gram gave 18.3 c.c. 0.1 normal $NH_4OH$; 0.3540 gram gave 23.0 c.c. 0.1 normal $NH_4OH$, indicating 9.27 per cent. and 9.09 per cent. respectively or an average of 9.18 per cent. of nitrogen in the opaque egg jell.

Almost no chemical work has been done with the amphibian egg jells aside from the observation that the nitrogen content is low due to the presence of a carbohydrate nucleus. Of course the above results do not give the true nitrogen content of the egg jell for they should have been corrected for ash content, but this was not possible due to lack of material. The figures do show, however, that the jells differ chemically as well as in appearance, and that the difference in nitrogen content is in the same direction and of almost precisely the same amount that it would be if the opaque appearance were produced by an admixture of albumen (nitrogen = 15 per cent.–16 per cent.) with the normally occurring mucins.

Station for Experimental Evolution,
August 17, 1914.

# THE RELATION OF THE BODY TEMPERATURE OF THE EARTHWORM TO THAT OF ITS ENVIRONMENT.

CHARLES G. ROGERS AND ELSIE M. LEWIS.[1]

In a paper by one of us upon the "Temperature Coefficient of the Rate of Heart Beat in Certain Living Animals"[2] the assumption was made that the temperature of the living animal (worm or fish-embryo) under observation corresponded very closely to that of the water surrounding it. The same assumption has been made by other workers in this field, *e. g.*, Snyder[3] in his work upon the isolated heart of the Pacific terrapin assumed that the temperature of the more or less bulky heart muscle of the terrapin was conditioned by the temperature of the solution in which it was placed. Robertson[4] also in his work upon *Ceriodaphnia* assumed that the temperature of the water definitely represented the temperature of the tissues with which he was particularly concerned. Even more recently Loeb and Ewald[5] make use of the same assumption.

There are to be found in the literature of physiology statements concerning the body temperatures of the so-called cold-blooded animals, and an examination of the data offered reveals the fact that the various investigators who have taken the trouble to make any examination of the actual conditions find that the temperatures of the animals studied vary considerably from the temperatures of the surroundings. It is also true that many

[1] From the Department of Zoology, Oberlin College.

[2] Rogers, Charles G., "Studies upon the Temperature Coefficient of the Rate of Heart Beat in Certain Living Animals," *American Journal of Physiology*, 1911, Vol. 28, No. II., pages 81–93.

[3] Snyder, C. D., "On the Influence of Temperature Upon Cardiac Contraction and Its Relation to Influence of Temperature upon Chemical Reaction Velocity," University of California Publications, Physiology, Vol. 2, No. 15, 1905.

[4] Robertson, T. B., "Note on the Influence of Temperature Upon the Rate of the Heart Beat in a Crustacean (*Ceriodaphnia*)," BIOLOGICAL BULLETIN, 1906, Vol. X., No. 5, pp. 242–248.

[5] Loeb, Jacques and Ewald, W. F., "Die Frequenz der Herztätigkeit als eindeutige Funktion der Temperatur," *Biochemische Zeitschrift*, 1913, Bd. 58, H 3, 177–185.

of the observations recorded were made by means of mercurial thermometers, though some were made by thermocouples with galvanometers. In reading the statements of the different investigators one is left with the feeling that with the more improved appliances of the present day one ought to be able to make observations which would be more satisfactory than those published. The following table summarizes the results of a number of investigators, and is compiled from data quoted by Milne-Edwards.[1]

| Animal. | | Authority. |
|---|---|---|
| Fishes | less than 1°C. above surrounding water | Milne-Edwards |
| Frog | 0°.32–2°.44 " " " | Czermak |
| Frog | 0°.0 –0°.575 " " " | Becquerel |
| Frog | 0°.04 " " " | Dutrochet |
| Toad | 0°.2 " " " | " |
| Frog | 0°.7 –0°.3 " " " | Dumeril |
| *Proteus* | 1°.25 " " " | Rudolphi |
| *Proteus* | 2°.6 –5°.6 " " " | Czermak |
| Crayfish | 6°.0 " " " | Rudolphi |
| *Maia sq.* | 0°.3 –0°.9 " " " | Valentin |
| *Limax* | 0°.33–0°.50 " " " | Spallanzani |
| Snail | 2°.0 " " " | Hunter |
| Snail | 1°.1 " " " | Martine |
| Snail | 0°.9 " " " | Becquerel |
| Snail | 1°.5 –2°.0 " " " | Schnetzler |
| *Aplysia* | 0°.1 –0°.8 " " " | Valentin |
| *Octopus* | 0°.2 –0°.6 " " " | " |
| *Eledone* | 0°.9 " " " | " |
| Annelids | 0°.56–0°.85 " " " | Hunter |
| *Lumbricus* | 1°.11–1°.39 " " " | " |
| Holothuria | 0°.2 –0°.6 " " " | Valentin |
| Ophiurian | 0°.3 " " " | " |
| *Asterias ru.* | 0°.6 " " " | " |
| Sea Urchin | 0°.4 –0°.5 " " " | " |
| *Medusa Pelagia* | 0°.2 –1°.0 " " " | " |
| *Medusa Cassiopea* | 0°.3 " " " | " |
| Actinians | 0°.2 –0°.5 " " " | " |

In the table it will be noted that the temperatures determined for the different animals show, for the most part, rather small variations from that of the surrounding water. In a few cases the variation is quite considerable, and appears to make desirable a reëxamination of the facts. This is especially true in view of

[1] Milne-Edwards, "Leçons sur la Physiologie et L'Anatomie Comparée de L'Homme et des Animaux," T. VIII., Paris, 1863.

the fact that certain scientific friends have raised question as to the validity of the assumption upon which the temperature coefficient work was based. It is with an idea of attempting to answer any questions as to the propriety of assuming that the temperature of the earthworm is represented by the temperature of the surroundings, that the present investigation is here reported.

## Methods.

A method of measuring differences of temperature by means of the electromotive force developed when the junctions of wires of different metals of a common circuit are not at the same temperature was described by Nobili and Melloni[1] about 1830. Since that time galvanometers have been made more sensitive, and it has also been made possible to obtain pure metallic wires of small diameter, and of small heat capacity. The authors named were the first to apply this method of temperature measurement to living animals, and now that the methods of use have been somewhat improved the same method has been employed for the measurement of the amount of heat given off in a given contraction of a frog muscle.[2] The method can be made accurate enough to measure differences as small as 1/150° C. For the purpose of the investigation here reported it did not seem necessary to make measurements as small as those recorded by Hill, so the number of junctions of the wires was not at all increased. The thermo-couples used consisted of No. 32 copper and No. 32 constantan wires joined together as shown in Fig. 1. In some of the couples the wires were simply twisted together and in others the junction was made secure by a small drop of solder. We were not able to determine that for our purpose the soldered junctions were any more efficient than those not soldered. The junction used within the body of the worm was mounted within a slender glass tube in such a way as to have the two wires of the couple thoroughly insulated from each other except at the junction point (Fig. 2). This was accomplished by placing

[1] Nobili et Melloni, "Recherches sur plusiers phenomenes calorifiques enterprises au moyen du thermo-multiplcaiteur," *Ann. de chimie et de physique*, 1831, T. XLVIII., p. 208.

[2] Hill, A. V., "The Energy Degraded in the Recovery Process of Stimulated Muscles," *Journal of Physiology*, 1913, Vol. 46, pp. 28–80.

one of the wires in a finely drawn glass tube, allowing the end of the wire to extend slightly below the end of the tube, where it was twisted together with the other wire of the couple. The small glass tube with the two wires was then placed inside another glass tube of a diameter just sufficient to receive them easily. The glass of the outer tube was then sealed over the junction of the wires, and the whole bent into a convenient form for handling. The upper open ends of the tubes were sealed with wax to prevent the access of any water and the apparatus was ready for use.

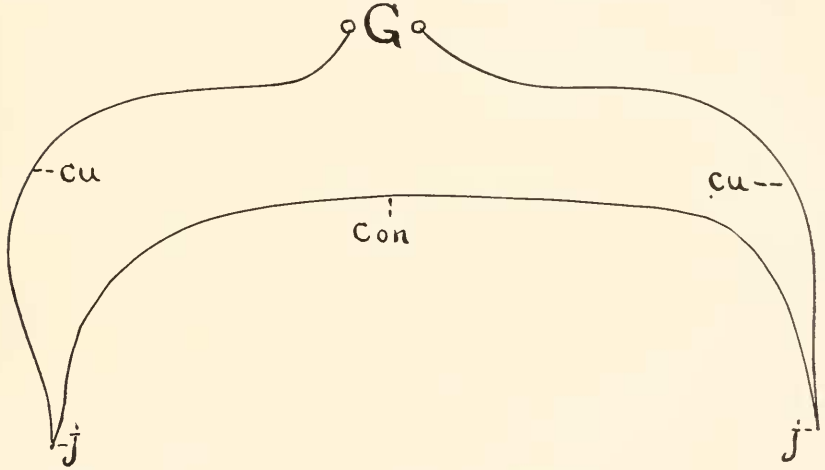



FIG. 1. Diagrammatic scheme of thermocouple. *G* = galvanometer; *cu* = copper wire; *con* = constantan wire; *j* = junction.

The galvanometer used in this work was one of the D'Arsonval type made by Gaertner. It was provided with a dead beat coil, and was so heavily damped that it was found desirable to allow two or three minutes for the galvanometer mirror to come to rest when making observations. It was found that with the scale at about 1 meter distance 1° C. difference in the temperature of the two junctions of the thermo-couple was represented by a shifting of the reading of the galvanometer scale by about 16 millimeters. The actual amount of shift varied somewhat from day to day from this value, and was re-determined for each day's work.

The technic of determining the temperature of the interior of

the worm was very simple. It consisted in placing the glass-covered junction of the thermocouple in the mouth of the worm, and carefully pushing it down through the œsophagus, crop and gizzard until it came to lie in the stomach intestine. The other junction of the couple was placed in water of a known temperature, and the two end wires of the thermocouple were attached to the galvanometer. The temperature of the water in which the free junction of the thermocouple was placed was determined by a Beckman thermometer which had been set to a definite reading by the side of a certified standard thermometer. The temperature of the water was determined to 1/100° C., and was noted for every observation so that in case of any variation in the temperature of the water we should be able to make the necessary correction in the results. The temperature of the worm was shown by the amount of the deviation of the galvanometer reading, from the zero reading, divided by the number of millimeters representing 1° C., and adding this amount to the known temperature of the water. In this way we were able to make readings which could be accepted as accurate to within 0.03° C., which for our purpose seemed to be sufficient.

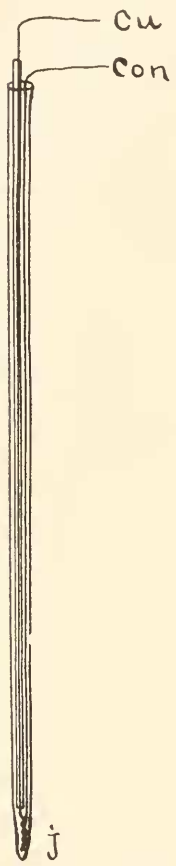



FIG. 2. Sketch of thermo-electric clinical thermometer for use with the earth-worm. *cu* = copper wire; *con* = constantan wire; *j* = junction.

The following table gives data derived from three experiments to show how closely and how rapidly the temperature of the worm becomes adjusted to the temperature of the water in which it is immersed. We have not thought it necessary to multiply examples as all the facts observed are in perfect harmony with those offered. In the experiments here cited both the worm and the free junction of the couple were placed in the same bath. The difference between the zero and in-circuit readings of the

galvanometer would then indicate the difference in temperature between the temperature of the water and the interior temperature of the worm if any such difference exists. After each reading the worm and free junction of the couple were placed in the water of the temperature indicated for the next following reading.

| | Time. | Temp. of Water in Which Worms Were Placed. | Zero Reading of Galvanometer. | Galvanometer Reading Couple in Circuit. | Difference in Millimeters. | Temperature of Worm. |
|---|---|---|---|---|---|---|
| *A.* | 9.40 | 11.40° C. | 345 | 345 | 000 | 11.40° C. |
| | 10.10 | 21.30 | 345 | 345 | 000 | 21.30 |
| *B.* | 9.00 | 21.24 | 403 | | | |
| | 9.17 | 21.24 | 403 | 402 | 001 | 21.30 |
| | 9.25 | 10.00 | 403 | 403 | 000 | 10.00 |
| | 9.35 | 21.20 | | | | |
| | 9.40 | 21.20 | 408 | 408 | 000 | 21.20 |
| | 9.55 | 13.00 | 408 | | | |
| | 10.00 | 13.00 | 408 | 408 | 000 | 13.00 |
| *C.* | 10.30 | 21.20 | 403 | 404 | 001 | 21.14 |
| | 10.40 | 17.00 | 403 | 404 | 001 | 17.94 |
| | 10.45 | 21.20 | 403 | 404 | 001 | 21.14 |

The above data are representative of a large number of observations made during an investigation upon the effect of temperature changes upon the rate of contraction of the dorsal blood vessel of the earthworm, *Lumbricus agricola*, and indicate very clearly that the animal under investigation adapts itself with remarkable quickness and closeness to the temperature of its environment. In fact we think it may safely be said that the worm will adapt itself to a change of at least ten degrees Centigrade within two minutes, to an accuracy of 0.05° C. This fact makes it possible then to use the temperature of the water surrounding the animal as an indicator of the temperature of the animal, in the case of the earthworm, for experiments upon the temperature coefficient of heart action, and assures us that the worm need not be subjected to a bath of a given temperature for any great length of time in order to get an accurate result. It is very likely true that the same principle will be found to hold good for other animals of a similar kind and habit, certainly for marine worms, fish embryos, small crustacea, etc. It is the purpose of the authors to continue the investigation upon other

forms in order to determine to what extent we may be at liberty to assume the temperature of the surrounding fluid to be an indicator of the temperature of the tissues.

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# THE TEMPERATURE COEFFICIENT OF THE RATE OF CONTRACTION OF THE DORSAL BLOOD-VESSEL OF THE EARTHWORM.

CHARLES G. ROGERS AND ELSIE M. LEWIS.[1]

Certain unpublished criticisms of a previous paper of the senior author[2] have led us to make a further investigation of the effects of changes of temperature upon the rate of contraction of the dorsal blood vessel of the earthworm. The criticism offered against the previous work was that no evidence was presented to show that the temperature of the forms studied, worms and fish-embryos, was the same as that of the water in which they were immersed. The force of the criticism is recognized, and we are now able to present the results of an investigation in which the temperature of the worm studied was determined by means of a delicate clinical thermometer inserted in the long, tubular, alimentary canal of the worm.

We are now publishing under another title[3] an account of the work in which it is shown that the temperature of the surrounding water does furnish an excellent indicator of the inner body temperature of the earthworm, when this animal is immersed in water for experimental purposes. Inasmuch as this is true we have no doubt that the previous work upon *Nereis*, *Tubifex*, and the embryos of *Fundulus* and the toad-fish will bear the same sort of inspection.

We will not at this time take up any discussion of the literature of the subject. The papers of Snyder, Robertson, Loeb and Ewald and others are available for examination. The formula employed for the computation of the temperature coefficients

[1] From the Department of Zoology, Oberlin College.

[2] Rogers, Charles G., "Studies Upon the Temperature Coefficient of the Rate of Heart Beat in Certain Living Animals," *American Journal of Physiology*, 1911, Vol. XXVII., pp. 81–93.

[3] Rogers, Charles G., and Lewis, Elsie M., "The Relation of the Body Temperature of the Earthworm to that of its Surroundings," BIOLOGICAL BULLETIN, 1914, Vol. XXVII., pp. 261–267.

is the one used by Snyder in all his work, and concerning which he has presented some matters of historical interest,[1]

$$Q_0 = \left(\frac{K_1}{K_0}\right)^{\frac{10}{T_1 - T_0}}.$$

The material used in this study was the large earthworm, *Lumbricus agricola*. This form presents two desirable features for this work; 1st, the animals are not by nature restricted to any definite or narrow limits of temperature at which their normal physiological processes take place; and 2d, it is well adapted structurally for the work in that it is easily possible to place a delicate temperature measuring apparatus in the alimentary canal, and it is also easy to see the contractions of the dorsal blood vessel through the more or less transparent body wall. This last fact is of special importance as it makes it practicable to leave the animal undisturbed in its constant temperature bath, thus obviating any disturbance of the vascular contractions through nervous action due to stimulation from without.

## Methods.

As a preliminary to the actual temperature work a number of worms were subjected to immersion, for varying periods, in water to ascertain what harmful effects might result. Without going into detail as to this work it may be stated that the worms are able to withstand immersion in tap water for a sufficient time to allow all the experimentation needed for the temperature studies, without showing any harmful effects. In fact certain worms have been immersed for as long a period as two weeks without showing any injurious effects.

The temperatures of the worms were regulated, then, by placing them in baths of water, the temperature of which was controlled by placing the dishes in thermostats having practically constant temperatures, in refrigerators cooled by ice, or in the running water of the laboratory, which was found to have a very constant temperature.

The temperatures of the worms were determined by means of

[1] Snyder, C. D., "On an Interpolation Formula Used in Calculating Temperature Coefficients for Velocity of Vital Activities, Together with a Note on the Velocity of Nerve Conduction in Man," Science, N.S., Vol. XXXIV., No. 874, p. 415.

delicate clinical thermometers, in the form of thermo-couples, which could be inserted into the mouths of the worms and pushed on down into the stomach intestine. These thermo-couples were made of No. 32 copper and No. 32 constantan wires. Any difference in the temperature of the two junctions of the couple sets up an electromotive-force proportional in its strength to the amount of the temperature difference, and which can be accurately measured by means of a delicate galvanometer. In this particular work it was found that 1° C. was represented by a shift in the reading of the galvanometer scale of about 16 mm. As it was practicable to read to half millimeters it will be seen that a temperature difference of 0.03° could be determined. For a somewhat more detailed account of the method of temperature measurements the reader is referred to another paper.[1]

The worm having been subjected to a given temperature for a sufficient length of time to have become completely adjusted to the new condition, the temperature of the worm was noted, and the length of time required for a definite number of beats of the dorsal blood-vessel, usually 25, was taken by means of a stop-watch reading to fifths of a second. The worm was then changed to another bath at a different temperature, allowed to remain long enough to become thoroughly adjusted to the new condition, and another reading of the rate of contraction of the dorsal blood vessel made. From the data thus obtained the temperature coefficient of the rate of contraction was calculated by means of the formula referred to above.

It may be said that all possible precautions were taken to avoid serious errors in the work. Temperatures were determined by making the readings of the galvanometer against a certified thermometer calibrated to tenths of a degree C. The temperatures of the various baths were kept as uniformly constant as possible and the exact temperature taken each time a count was made. Occasionally one is in doubt as to whether a contraction of the dorsal blood vessel has actually taken place. In such a case the reading was thrown out and another made so as to make sure of the fact. The temperature of the room was kept as constant as possible in order to avoid any changes of resistance

[1] Rogers, Charles G., and Lewis, Elsie M., *l. c.*

in the copper wires and in the galvanometer which might tend to disturb the results. Manipulation of the worms was reduced to a minimum in order that nervous effects might not be introduced to invalidate the temperature effects. Reading of the rates of contraction of the dorsal blood vessel before and after the insertion of the glass covered junction indicated that the mere insertion of the instrument in the alimentary canal made no difference with the rate.

TABLE I.

| No. of Worm. | $K_1$ | $K_0$ | $T_1$ | $T_0$ | $Q_{10}$ |
|---|---|---|---|---|---|
| 3 | 19.35 | 13.74 | 21.43° | 11.60° | 1.406 |
| 5 | 15.00 | 9.80 | 18.00° | 11.50° | 1.925 |
| 6 | 20.35 | 10.90 | 18.00° | 11.50° | 2.593 |
| 7 | 21.10 | 13.80 | 16.04° | 11.50° | 2.548 |
| 8 | 15.72 | 8.92 | 16.04° | 11.50° | 2.096 |
| 9 | 22.00 | 9.93 | 16.04° | 11.50° | 5.766 |
| 10 | 16.93 | 10.60 | 16.04° | 11.50° | 2.805 |
| 11 | 17.75 | 11.29 | 16.04° | 11.50° | 2.642 |
| 12 | 14.79 | 11.76 | 16.04° | 11.50° | 1.656 |
| 14 | 27.00 | 19.40 | 26.40° | 11.70° | 1.272 |
| 16 | 26.90 | 14.92 | 26.40° | 11.60° | 1.485 |
| 17 | 15.27 | 11.13 | 26.50° | 11.60° | 1.236 |
| 18 | 19.70 | 15.33 | 26.50° | 11.60° | 1.176 |
| 19 | 29.30 | 12.50 | 27.13° | 12.55° | 1.793 |
| 21 | 24.15 | 13.50 | 27.12° | 12.55° | 1.280 |
| 24 | 33.60 | 24.00 | 27.00° | 16.36° | 1.372 |
| 26 | 37.80 | 26.70 | 26.60° | 24.60° | 5.754 |
| 27 | 32.56 | 19.35 | 20.00° | 9.50° | 2.044 |
| 28 | 35.27 | 24.55 | 20.00° | 9.50° | 1.413 |
| 29 | 21.39 | 8.85 | 27.50° | 14.00° | 1.621 |
| 30 | 28.20 | 12.13 | 27.10° | 14.00° | 1.899 |
| 31 | 20.40 | 13.18 | 27.70° | 14.00° | 1.365 |
| 32 | 27.48 | 11.75 | 27.30° | 14.04° | 1.898 |
| 33 | 21.50 | 12.66 | 26.50° | 14.02° | 1.525 |
| 34 | 26.55 | 14.05 | 25.60° | 14.03° | 1.733 |
| 35 | 22.00 | 12.95 | 25.08° | 13.88° | 1.605 |
| 36 | 24.86 | 12.63 | 25.08° | 13.92° | 1.838 |
| 37 | 16.97 | 8.95 | 22.54° | 13.95° | 2.109 |
| 38 | 20.40 | 11.58 | 22.46° | 13.96° | 1.947 |
| 39 | 22.43 | 11.86 | 21.86° | 13.94° | 2.236 |
| 40 | 21.59 | 9.81 | 21.43° | 13.95° | 2.871 |
| 41 | 26.80 | 11.16 | 22.90° | 13.72° | 2.603 |
| 42 | 21.97 | 11.10 | 22.95° | 13.74° | 2.099 |
| 43 | 23.33 | 11.94 | 22.71° | 13.80° | 2.121 |
| 44 | 25.00 | 11.23 | 23.18° | 13.73° | 2.388 |
| Average value of temperature coefficient for all specimens..... | | | | | 2.173 |

The preceding table, Table I., gives the data derived from the actual experiments and also the values of the temperature coefficient calculated from the data. In the tables the letters

$K_1$ and $K_0$ indicate the rates in contractions per minute at the temperatures $T_1$ and $T_0$ respectively, in degrees Centigrade.

If we arrange the data of Table I. so as to show the relation existing between the higher and lower temperature ranges and the value of $Q_{10}$ we have evidence that for the lower temperatures there is a higher coefficient than for the higher ranges. This fact is not so clearly shown in the case of the earthworm as in some of the forms already studied, probably for the reason that it is very difficult to count the beats of the dorsal blood vessel of the earthworm at temperatures below 8° C. The movements of the walls are so feeble and so slow that one is not sure when a contraction has taken place.

TABLE II.

| $T_1$. | $T_0$. | Number of Worms. | Average $Q_{10}$. |
|---|---|---|---|
| About 27° to about | 14° | 4 | 1.671 |
| " 27° " " | 12° | 2 | 1.536 |
| " 26° " " | 11° | 4 | 1.292 |
| " 25° " " | 13° | 3 | 1.725 |
| " 22° " " | 13° | 6 | 2.199 |
| " 21° " " | 13° | 2 | 2.533 |
| " 20° " " | 9° | 2 | 1.728 |
| " 16° " " | 11° | 6 | 2.919 |

In Table II. we have such an arrangement of the data as suggested in the preceding paragraph. It will be noted that while in general the coefficients for the lower ranges are higher than those for the higher ranges of temperature, there are very marked exceptions to the rule. The only explanation we have to offer at this time for the rather marked variation from what we should expect, if the beat of the dorsal blood vessel of the earthworm is subject to the same laws as the beats of various hearts already studied, is that we have here to deal with a series of nervous effects which must be in some way eliminated in order to avoid complications. Up to the present time we have not found any means of avoiding these nervous effects in the worm, though in fish embryos where the rate of heart beat was studied before the nervous connections were established it was found that one could predict with some degree of certainty what the rate of heart contraction should be at any stated temperature.

That the rate of beat of the dorsal blood vessel of the earthworm is to some extent under the control of the nervous system will be shown in another publication from this laboratory.

It is to be noted also that the temperature coefficient of the rate of contraction of the dorsal blood vessel of the earthworm is, for the temperatures at which the worm would naturally live, of the same general magnitude as those of chemical reactions, and the average for the whole series, 2.173, also falls within the limits usually set for the temperature coefficients of such reactions. We have no reason, as yet, to assign for the much reduced coefficients for the higher temperature ranges.

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# AUDITORY POWERS OF THE CATOCALA MOTHS; AN EXPERIMENTAL FIELD STUDY.[1]

C. H. TURNER AND E. SCHWARZ.

## Historical Resumé.

Near the close of the nineteenth century, Romanes ('91) wrote: "Among insects organs of hearing certainly occur, at least in some, although the experiments of Sir John Lubbock appear to show that ants are deaf. The evidence that some insects are able to hear is not only morphological, but also physiological, because it is only on the supposition that they do that the fact of stridulation and other sexual sounds being made by certain insects can be explained, and Brunelli found that when he separated a female grasshopper from the male by a distance of several meters, the male began to stridulate in order to inform her of his position, upon which the female approached him. I have myself published observations proving the occurrence of a sense of hearing among the Lepidoptera."

The tone of three fourths of the above paragraph is characteristic of practically all of the early works upon the auditory powers of insects. Those men were convinced that insects hear; not because they had experimentally demonstrated it, but for morphological reasons, and because many kinds of insects can produce sounds. They believed that an insect would not be endowed with the power of producing sounds unless the other members of the species could hear. At first in the Orthoptera and later in other groups of insects, peculiar organs were found; consisting essentially of vibratory hairs attached to certain cells that seem to be sensory in nature. In some cases these hairs are in cavities and in others they are not. Such was the nature of the work of Siebold ('44), Leydig ('55), Henson ('66), Lee ('83, '85), Graber ('75, '82), Weinland ('91), Adelung ('92) and others. As late as 1905 Radl expressed the following thought. No matter

[1] For the identification of the species and for the experimental work on *C. unijuga*, E. Schwarz alone is responsible; the field work was performed jointly; for the planning of the work, for the historical resume and for the method of treatment, C. H. Turner is solely responsible.

how often in recent years doubt has arisen as to the ability of insects to hear, it has been largely maintained that they possess an auditory sense, and for the following two reasons: (1) the ability of many insects to produce sounds as a part of their normal behavior; (2) the possession by insects of organs which structurally seem fitted to act as receptors of sound waves.

Students interested in the morphological method of investigating this question will find the paper by Radl ('05) intensely interesting. After epitomizing the work by Lee ('83, '85), by Graber ('82), and by Weinland ('91), he states that, on a priori grounds, he doubted the assumptions of Graber; but that certain experiments had convinced him that insects have a crude auditory sense. In support of his contention, he offers the following reasons.

1. Graber is inaccurate when he claims that the chordotonal organs are located rigidly between two immovable parts of the body; for the proximal end is attached to an indifferent part of the body, while the distal end is in close proximity to one or more muscles.

2. The chordotonal organ cannot function like a string attuned to a certain pitch; because it expands and contracts.

3. All of the chordotonal organs examined are attached at each end to a chitinous framework and the nerve penetrates from the side.

4. The chordotonal organs resemble somewhat those muscles which occur especially in the limbs of the Arthropoda—muscles which terminate in long tendons.

5. No chordotonal organ is found in either the Myriapoda or the Arachnida.

6. Chordotonal organs are found in some insects for which a sense of hearing could have no significance. They are well developed in caterpillars; even in those of the Tortricidæ, which spend the entire larval period inside of some fruit. They are also well developed in the internal parasites of certain insects.

7. All attempts to determine experimentally that insects react to pure and simple tones have yielded negative results: however, it is comparatively easy to evoke responses of insects to shrill noises, such as the voice of a cricket or the screech produced by

drawing a file across the edge of an iron or a glass plate. This is not a tactile reaction.

8. There is no evidence that noise, as such, causes the orientation of insects. The sounds produced by insects are more an outburst of inner feeling than an attempt to entice the female by the male.

9. The outcome of the whole matter is that there is an auditory sense in insects; but, it is on a much lower plane of development than that of the vertebrates. Its anatomical and physiological antecedents are to be found, not in the tactile organs and contact activities; but, anatomically in sense organs which register muscle activities and physiologically in general sensation (Gemeingefuhlen). The auditory sense of insects is a highly refined muscular sense.

Although the work of the early investigators was largely, often entirely, morphological, it must not be concluded that no experimental work has been done on the auditory powers of insects and their near kin. Such experiments have been performed in several groups of insects; but the results are inharmonious. Buttel-Reepen ('00) and De Fraviere believe that bees can hear. Buttel-Reepen's statement is based upon his observation that bees respond in a definite manner to the sounds of their own kind.

Huber ('10) and Forel ('03) interpret their experiments to mean that ants cannot hear. Lubbock's experiments likewise yielded negative results; yet, in spite of this, he was unwilling to admit that ants, wasps and bees cannot hear. Weld ('99) thinks he has experimentally demonstrated that ants can hear. Fielde and Parker ('04) interpret their experiments to mean that ants do not respond to sound vibrations as such. C. H. Turner ('07) is equally positive that his experiments demonstrate that ants can hear. At one time Wheeler believed, not only that ants can hear; but that they communicate by means of sounds; but, after the appearance of the paper by Fielde and Parker, he ('10) asserts that there is not sufficient evidence to warrant the assumption that ants can hear.

E. A. Andrews ('11) is convinced that termites hear. He writes: "In a community suspended from the ceiling by a copper

wire and represented by many thousands on a moist block of artificial stone which they got to from the nest by means of a long stick as bridge, it was first observed by Mr. Middleton that the noise of thunder and of blasting rocks was followed by a quick and very remarkable departure of almost all of the termites towards the nest. The blocks of stone weighed some sixteen pounds each and rested in a large pan of water on a firm wall of stone, so that it seemed likely that the concussion of the air came to the termites directly and not as a tremor of the stones they were clustered on. The same precipitous flight of the multitude of termites from these stones to the nest along the bridge was brought about by dropping a board upon the concrete floor with a loud crash. Even the clapping of hands, which probably shook the stone foundation but imperceptibly, served to drive the termites back to the nest. . . . Attempts to influence the termites by blowing horns of various pitches near them failed though considerable disturbance of the air was produced."

Montgomery ('10), after reviewing all that had been written on the auditory powers of spiders, concludes that spiders are deaf.

The above is not an exhaustive discussion of the published research work on the auditory powers of insects other than the Lepidoptera; yet, we trust it is sufficient to show the inharmoniousness of the results of different investigators.

To the best of our knowledge, the first published results of experiments upon the auditory powers of butterflies and moths is an article by Romanes ('76) which appeared about thirty-six years ago. The portion referring to the Lepidoptera is so short that we quote it in full. "It seems worth while to add a few words with respect to the sense of hearing in insects. So far as I am aware, the occurrence of such a sense in this class has never been actually proved. Although on *a priori* grounds there can scarcely be any doubt concerning the fact of some insects being able to hear; seeing that in so many species stridulation and other sounds are made during the season of courtship. In the case of moths, however, I believe that sounds are never emitted—except, of course, the death's head moth.[1] It therefore becomes inter-

[1] Romanes was mistaken when he asserted that the death's head moth is the only Lepidopteran than produces sounds; for the literature contains records of

esting to observe that an auditory sense is certainly present in these insects. Several kinds of moths have the habit of gently, though very rapidly, vibrating their wings, while they themselves are at rest on a flower or other surface. If, while this vibrating movement of the wings is going on, the observer makes a sudden shrill note with a violin, or fife, etc., the vibrating movement immediately ceases, and sometimes the whole body of the insect gives a sudden start. These marked indications of hearing I found invariably to follow a note with a high pitch, but not a note with a low one."

Heinrich ('09) remarks that collectors using a net to capture *Limenitis populi* and *Catocala fraxini* have observed that the insects often take flight before a collector is near enough to capture them. Accordingly to him no conclusive evidence has been published on the sense of hearing of insects, especially of the Lepidoptera. He noticed a *Laurentia suffumata* alight in a concert pavilion and remain quietly while the brass band played three selections, one of which was Wagner's Götterdammerung. He also noticed that certain Lepidoptera were more easily approached at twilight than when the sun was shining brightly, and he could not understand why this should be true if they were warned by a sense of hearing. He is convinced that, in all of these cases, it is vision, not audition, that warns butterflies and moths of the approach of man.

Hamann ('09) was led to investigate this subject by the remarks of collectors that butterflies and moths undoubtedly hear. One collector remarked that the noise caused by removing the cork from the cyanide bottle often caused these insects to fly. To this Haman replies that since the net is usually placed beneath the insect before the cork is removed it is probable that the sight of the net caused the flight. To test the matter, the following experiments were performed by him. (1) He approached a tree in such a manner as to be invisible to an *Apatura iris* L. resting thereon, and struck the tree with the bamboo handle of his

several species of Lepidoptera that emit sounds. Indeed, scarcely had his article appeared before several of his contemporaries published, in *Nature*, protests in which were cited several examples of sound-producing Lepidoptera. Recently Omensetter ('12) and Stephan ('12) have described several sound-producing forms of butterflies and moths.

collecting net. To this the insect made no response; but, as soon as the net approached it took flight. (2) a repetition of the experiment with *Vanessa* yielded similar results. (3) He noticed that certain Catocalas, which were not disturbed by the noise of a passing automobile, flew upon the approach of man. These experiments convinced him that butterflies and moths cannot hear.

Deegener's work ('09) is morphological. Between the thorax and the abdomen, on the ventral side of the body of all species of Nocturidæ, there is a depression containing chitinous structures and hairs which are connected with what seem to be sensory cells. A careful examination of this organ in *Pseudophia lunaris* convinced Deegan that it is probably an auditory organ.

Rothke ('09) confined a *Limenitis artemis* in a cage which he placed on the top of a pedestal two feet high. The sides and back of this cage were constructed of wood; but the front was covered with wire fly netting. At nine P.M., while the front of the cage was illuminated by means of a kerosene lamp, Rothke stepped to one side and made a slight noise. To this the insect made no response. The investigator then tapped rapidly and sharply upon the floor with a leather slipper. Although the jar was not sufficient to shake the stand upon which the cage rested, and although the investigator could not be seen by the insect, yet it slowly raised its wings until they met above its back and then lowered them again. Several repetitions of this experiment yielded identical results. During the intervals between the experiments the creature remained immobile. After the moth had been quiet for one quarter of an hour, Rothke rapped upon the table with a tumbler. Immediately the insect flapped its wings. About midday he discovered a specimen of *Catocala unijuga* resting quietly, about six feet from the ground, upon a pine tree one and a half feet in diameter. He picked up a stone about fifteen centimeters in diameter and threw it against the tree-trunk. Although the moth could not see the stone and although the blow was too slight to jar such a large tree, yet the moth flew away. Rothke is convinced that butterflies and moths can hear.

Observations made upon *Catocala pacta* L. convinced Richter

('09) that the crackling of twigs under his feet and even the slight noise made by removing the cork from his collecting bottle disturbed this moth. In another article, the same investigator ('10) has made a comparative study of the auditory powers of day-flying and night-flying Lepidoptera. He investigated the day-flyers; *Apatura* sp., *Vanessa* sp., *Limenitis populi* L., and *Sat. alcyone* Schiff. He found that species of *Apatura* and of *Vanessa* made no responses to sound so long as no visible object disturbed them. During a severe storm, he noticed a number of *Sat. alcyone* perching on a limb. Neither whistling, nor the clapping of hands, nor shaking of the limb had any effect on them: but, as soon as the hand of the collector approached them, they flew. A *Vanessa antiopa*, resting on a telegraph pole, was not disturbed by the shrill whistle and the rumbling noise of a passing train. In studying *Catocala fraxini* L., a night-flying moth, he noticed that it made no responses to the noises made by wagons, automobiles and the bells of a ferry; but that it responded readily to slight, high-pitched, sounds. He argues that the failure of this moth to respond to the sounds made by wagons and such things is because such sounds have no life significance for the moth. On the other hand, the ready response to the other sounds mentioned is due to their similarity to sounds made by field-mice, bats and owls—sounds which for the moth have a pronounced life significance. Partly influenced by the knowledge that Geegener had discovered chordotonal organs in the Noctuidæ and more so by the observations just described, Richter is convinced that day-flying Lepidoptera are warned by visual and night-flying forms by auditory stimuli.

For years Rober ('10) has raised *Acerontia atropos* L. from pupae. Late one evening a female emerged and, before her wings were fully dried, a male emerged in the cage. In order to separate the two, the female was removed to a cage in the bottom of which there was a crack as wide as one's finger. These cages were three meters apart. In the morning the female, which had escaped from confinement, was found perched on the cage containing the male. A person who slept in the room with these two moths asserts that, for a long time that evening, those moths emitted sounds. Rober concludes that these were love calls and that the

moths mutually heard. In face of the well known fact that the sense of smell is well developed in butterflies and moths, the evidence just cited does not appear to be conclusive.

Is it possible for anyone to read the above historical resume and not be convinced that there is need for much exact experimentation upon the auditory powers of insects? Evidently the last word has not been spoken.

## Description and Discussion of Experiments.

Reading Stephan's ('12) recent articles on sound producing butterflies and moths induced in us the same thought that influenced the opinions of many of the early investigators; namely, animals that produce sounds as a part of their normal behavior can probably hear. We decided to make some crucial experiments. The Catocala moths were selected for the following reasons: (1) one of us is so well acquainted with the taxonomy of the group that it is easy to identify species afield; (2) the habit these moths have of resting during the day on some tree trunk and, when disturbed, flying to a nearby tree trunk renders them ideal material for field experiments.

## Preliminary Observations.

Observations afield taught us that there are certain sounds to which these moths do not respond. A favorite haunt of the Catocala moths of this vicinity is a small stretch of wood through which a railroad passes. Moths resting on trees near the tract are not disturbed by the whistle, rumble and roar of passing trains. Near that same place there is a pleasure garden in which the sounds of a noisy piano are often heard. No responses to the strains of the piano were noticed.

These observations are in harmony with those of Heinrich ('09), Hamann ('09) and Richter ('09, '10).

## Indoor Experiments on Catocala unijuga.

Three specimens freshly hatched from pupae were the subjects of these experiments. Each was kept in a separate room. Three times a day, for four days, the auditory powers of these moths was tested by whistling in a high key. Occasionally the moth was shielded from the draft of air caused by whistling;

## TABLE I.

*Catocala unijuga.* SPECIMEN NUMBER 1.

| Number of the Experiment. | Date. | Whistled, the Insect Shielded from the Air Currents. | Whistled, the Insect Not Shielded from the Air Currents. | Number of Times it Was Necessary to Whistle Before Response Was Received. |
|---|---|---|---|---|
| 1 | June 5, 1913, 10:00 A.M.... | *N* | | |
| 2 | " " " ... | | *F* | 1 |
| 3 | " " " ... | | *F* | 1 |
| 4 | " " " ... | *F* | | 4 |
| 5 | " " " ... | *Q* | | 5 |
| 6 | June 5, 1913, 10:30 A.M.... | | *F* | 2 |
| 7 | " " " ... | *Q* | | 6 |
| 8 | " " " ... | *F* | | 3 |
| 9 | " " " ... | *Q* | | 8 |
| 10 | June 5, 1913, 6 P.M..... | *F* | | 4 |
| 11 | " " " ..... | *Q* | | 3 |
| 12 | " " " ..... | *F* | | 6 |
| 13 | " " " ..... | *Q* | | 10 |
| 14 | June 6, 1913, 9:00 A.M..... | *N* | | |
| 15 | " " " .... | *Q* | | 4 |
| 16 | " " " .... | *F* | | 2 |
| 17 | " " " .... | *Q* | | 5 |
| 18 | June 6, 1913, 11.00 A.M.... | *F* | | 4 |
| 19 | " " " ... | *Q* | | 10 |
| 20 | " " " ... | *F* | | 4 |
| 21 | " " " ... | *Q* | | 15 |
| 22 | June 6, 1913, 6:00 P.M..... | *F* | | 8 |
| 23 | " " " .... | *Q* | | 15 |
| 24 | " " " .... | *F* | | 6 |
| 25 | " " " .... | *Q* | | 19 |
| 26 | June 7, 1913, 9:00 A.M..... | *F* | | 4 |
| 27 | " " " .... | *Q* | | 10 |
| 28 | " " " .... | *F* | | 4 |
| 29 | " " " .... | *Q* | | 6 |
| 30 | June 7, 1913, 10:00 A.M.... | *F* | | 3 |
| 31 | " " " ... | *Q* | | 8 |
| 32 | " " " ... | *F* | | 3 |
| 33 | " " " ... | *Q* | | 15 |
| 34 | June 7, 1913, 7:00 P.M..... | *F* | | 6 |
| 35 | " " " .... | *Q* | | 5 |
| 36 | " " " .... | *F* | | 4 |
| 37 | " " " .... | *Q* | | 6 |
| 38 | June 8, 1913, 10:00 A.M.... | *F* | | 2 |
| 39 | " " " ... | *Q* | | 10 |
| 40 | " " " ... | $N_1$ | | |
| 41 | " " " ... | *Q* | | 6 |
| 42 | June 8, 1913, 10:30 A.M.... | *N* | | |
| 43 | " " " ... | *Q* | | 6 |
| 44 | " " " ... | $N_2$ | | |
| 45 | " " " ... | *Q* | | 10 |
| 46 | " " " ... | *F* | | 2 |
| 47 | June 8, 1913, 6:00 P.M..... | *F* | | 4 |
| 48 | " " " .... | *Q* | | 3 |
| 49 | " " " .... | *F* | | 6 |
| 50 | " " " .... | *Q* | | 10 |
| 51 | " " " .... | *F* | | 13 |

Explanation of Tables I–III.

*N* indicates no response; *F* means that the moth flew; *Q* indicates that the insect waved its wings up and down or made some quivering movement.

## Table II.

*Catocala unijuga.* Specimen 2.

| Number of the Experiment. | Date. | Whistled, the Insect Shielded from the Air Currents. | Whistled, the Insect Not Shielded from the Air Currents. | Number of Times it Was Necessary to Whistle Before the Moth Responded. |
|---|---|---|---|---|
| 1 | June 6, 1913, 9:00 A.M..... | *Q* | | 6 |
| 2 | " " " .... | | *F* | 2 |
| 3 | " " " .... | *F* | | 5 |
| 4 | " " " .... | *Q* | | 4 |
| 5 | June 6, 1913, 10:00 A.M.... | | *F* | 1 |
| 6 | " " " ... | *F* | | 4 |
| 7 | " " " ... | *Q* | | 3 |
| 8 | " " " ... | *F* | | 2 |
| 9 | June 6, 1913, 6:00 P.M..... | *F* | | 1 |
| 10 | " " " .... | *Q* | | 3 |
| 11 | " " " .... | *F* | | 2 |
| 12 | " " " .... | *Q* | | 6 |
| 13 | June 7, 1913, 10:00 A.M.... | *F* | | 2 |
| 14 | " " " ... | | *F* | |
| 15 | " " " ... | *F* | | 4 |
| 16 | " " " ... | *Q* | | 5 |
| 17 | June 7, 1913, 11:00 A.M.... | *F* | | 2 |
| 18 | " " " ... | *QF* | | 4 |
| 19 | " " " ... | *F* | | 4 |
| 20 | " " " ... | *Q* | | 4 |
| 21 | June 7, 1913, 6:00 P.M..... | *F* | | 3 |
| 22 | " " " .... | *Q* | | 3 |
| 23 | " " " .... | *F* | | 2 |
| 24 | " " " .... | *Q* | | 8 |
| 25 | June 8, 1913, 9:00 A.M..... | *N* | | |
| 26 | " " " .... | | *F* | 1 |
| 27 | " " " .... | *Q* | | 3 |
| 28 | " " " .... | *F* | | 8 |
| 29 | June 8, 1913, 11:00 A.M.... | *F* | | 4 |
| 30 | " " " ... | *Q* | | 6 |
| 31 | " " " ... | *F* | | 4 |
| 32 | June 8, 1913, 7:00 P.M..... | *F* | | 10 |
| 33 | " " " .... | *Q* | | 6 |
| 34 | " " " .... | *F* | | 2 |
| 35 | " " " .... | *Q* | | 6 |
| 36 | June 9, 1913, 9:00 A.M..... | | *F* | 2 |
| 37 | " " " .... | *Q* | | 10 |
| 38 | " " " .... | *F* | | 2 |
| 39 | " " " .... | *Q* | | 10 |
| 40 | June 9, 1913, 11:00 A.M.... | | *F* | 2 |
| 41 | " " " ... | *Q* | | 9 |
| 42 | " " " ... | *F* | | 1 |
| 43 | " " " ... | *Q* | | 11 |
| 44 | June 9, 1913, 7:00 P.M..... | *Q* | | 6 |
| 45 | " " " .... | *F* | | 1 |
| 46 | " " " .... | *Q* | | 6 |
| 47 | " " " .... | *F* | | 10 |

## TABLE III.

*Catocala unijuga.* SPECIMEN NUMBER 3.

| Number of the Experiment. | Date. | Whistled, the Insect Shielded from the Air Currents. | Whistled, the Insects Not Shielded from the Air Currents. | Number of Times it Was Necessary to Whistle Before the Response. |
|---|---|---|---|---|
| 1 | June 7, 1913, 9:00 A.M..... | *N* | | |
| 2 | " " " .... | | *F* | 1 |
| 3 | " " " .... | *Q* | | 3 |
| 4 | " " " .... | *F* | | 2 |
| 5 | " " " .... | *Q* | | 10 |
| 6 | June 7, 1913, 10:00 A.M.... | *F* | | 2 |
| 7 | " " " ... | *Q* | | 6 |
| 8 | " " " ... | *F* | | 2 |
| 9 | " " " ... | *Q* | | 10 |
| 10 | June 7, 1913, 7:00 P.M..... | *F* | | 2 |
| 11 | " " " .... | *Q* | | 10 |
| 12 | " " " .... | *F* | | 4 |
| 13 | " " " .... | *Q* | | 15 |
| 14 | June 8, 1913, 9:00 A.M..... | *F* | | 3 |
| 15 | " " " .... | *Q* | | 6 |
| 16 | " " " .... | *F* | | 4 |
| 17 | " " " .... | *Q* | | 10 |
| 18 | June 8, 1913, 10:00 A.M.... | *N* | | |
| 19 | " " " ... | *Q* | | 10 |
| 20 | " " " ... | *F* | | 6 |
| 21 | " " " ... | *Q* | | 5 |
| 22 | June 8, 1913, 10:00 P.M.... | *F* | | 6 |
| 23 | " " " ... | *Q* | | 4 |
| 24 | " " " ... | *F* | | 4 |
| 25 | " " " ... | *Q* | | 10 |
| 26 | June 9, 1913, 9:00 A.M..... | *F* | | 4 |
| 27 | " " " .... | *Q* | | 3 |
| 28 | " " " .... | *F* | | 6 |
| 29 | " " " .... | *Q* | | 10 |
| 30 | June 9, 1913, 11:00 A.M.... | *F* | | 6 |
| 31 | " " " ... | *Q* | | 10 |
| 32 | " " " ... | *F* | | 5 |
| 33 | " " " ... | *Q* | | 8 |
| 34 | June 9, 1913, 6:00 P.M..... | *F* | | 10 |
| 35 | " " " .... | *Q* | | 2 |
| 36 | " " " .... | *F* | | 4 |
| 37 | " " " .... | *Q* | | 6 |
| 38 | June 10, 1913, 9:00 A.M.... | *Q* | | 1 |
| 39 | " " " ... | *N* | | |
| 40 | " " " ... | *Q* | | 6 |
| 41 | " " " ... | *F* | | 2 |
| 42 | " " " ... | *Q* | | 7 |
| 43 | June 10, 1913, 11:00 A.M... | *N* | | |
| 44 | " " " ... | *Q* | | 3 |
| 45 | " " " ... | *F* | | 1 |
| 46 | " " " ... | *Q* | | 11 |
| 47 | June 10, 1913, 6:00 P.M.... | *N* | | |
| 48 | " " " ... | *F* | | 2 |
| 49 | " " " ... | *Q* | | 6 |
| 50 | " " " ... | *F* | | 1 |
| 51 | " " " ... | *Q* | | 10 |

but, in some cases, the air current was allowed to strike the moth. The results of those experiments are recorded in tables I.–III.

On the twelfth of June the three specimens, which, up to that time, had been confined in separate rooms, were marked and placed in the same room. At nine A.M. that day, on the first sound of the whistle, they all flew, one after another, as though the flight of the first had evoked the flight of the others. At ten o'clock, the whistling caused two to fly and the other to quiver. The one that quivered was about ten feet away. On whistling again all flew. At six P.M. the whistling caused all to fly.

To our way of thinking this series of experiments is very instructive. That each of these three specimens responded to the whistle on the twelfth of June is unequivocal; that they usually responded to the whistle by either flying or by quivering is also evident; but, it is equally certain that two out of the three specimens did not respond to the whistle at all the first time it was sounded and that the third specimen responded in a feeble manner. When the moths did not respond to the blowing of the whistle at the beginning of the experiment, the current of air produced by whistling was allowed to strike the moth; immediately it flew, and thereafter it would usually fly when the whistle was sounded. There were some exceptions to this; but, in the main it was true. This seems a hint that the moth responds to sounds that have a life significance.

### Field Experiments.

These experiments were conducted in a small stretch of woods at Meramec Highlands, near St. Louis, Mo. Previous experience had taught us that these insects would not respond to loud sounds of low pitch. For that reason we used as the sound producing instrument a Galton whistle set to give a high shrill note. One of us would stand where the moth could be observed; but far enough away not to disturb it. Experience had taught us what would be a safe distance. The other, whistle in hand, would approach the tree on the opposite side to that on which the moth was resting. When this experimenter was near to the tree the whistle was held at about the level of the moth and sounded one or more times. In such a position it was absolutely impossible for the moth to see either the whistle or the experimenter. The

whistle was usually 180° from the moth; but occasionally it was placed ten to fifteen degrees away, but out of sight of the moth. In a few rare cases, for a special purpose, the whistle was blown in the presence of the moth. Whenever that was done it is indicated in the tables. The results of these experiments are recorded in Tables IV.–XI. The tables are self explanatory.

TABLE IV.

RESPONSES OF *Catocala flebilis* TO SOUND.

| Number of the Experiment. | Number of Times the Whistle Was Sounded. | Pitch of the Whistle. | Kind of Response. |
|---|---|---|---|
| 1 | 1 | $e^5$ | Flew. |
| 2 | 1 | | Flew. |
| 3 | 1 | | Flew. |
| 4 | 1 | | Flew. |
| 5 | 5 | | Flew. |
| 6 | 3 | | Flew. |
| 7 | 1 | | Flew. |
| 8 | 1 | | Flew. |
| 9 | 1 | | Flew. |
| 10 | 1 | | Flew. |
| 11 | 1 | | Moved its antenna, but did not fly. |
| 12 | 1 | | Flew. |

TABLE V.

RESPONSES OF *Catocala habilis* TO SOUND.

| Number of the Experiment. | Number of Times the Whistle Was Sounded. | Pitch of the Whistle. | Kind of Response. |
|---|---|---|---|
| 1 | 1 | $a^4$ | Flew. |
| 2 | 1 | | Flew. |
| 3 | 1 | | Flew. |
| 4 | 1 | | Flew. |
| 5 | 1 | | Flew. |
| 6 | 1 | | Flew. |

In the experiments recorded in this table (Table V.) the Galton whistle was held three feet from the tree on which the moth was resting.

TABLE VI.

RESPONSES OF *Catocala neogama* TO SOUND.

| Number of the Experiment. | Number of Times the Whistle Was Sounded. | Pitch of the Whistle. | Kind of Response. |
|---|---|---|---|
| 1 | 1 | $e^5$ | No response. |
| 2 | 1 | | No response. |
| 3 | 1 | $a^4$ | Flew (saw the whistle). |
| 4 | 1 | $a^4$ | Flew. |
| 5 | 1 | | Flew. |

## TABLE VII.

RESPONSES OF *Catocala piatrix* TO SOUND.

| Number of the Experiment. | Number of Times the Whistle Was Sounded. | Pitch of the Whistle. | Kind of Response. |
|---|---|---|---|
| 1 | 2 | $b^4$ | No response observed. |
| 2 | 1 | | Flew. |
| 3 | 1 | | Flew. |
| 4 | 1 | | Moved its wings up and down |
| 5 | 1 | | Ditto. |
| 6 | 1 | | Flew. |
| 7 | 1 | | No response observed. |
| 8 | 1 | | Moved its wings up and down |
| 9 | 1 | | Ditto. |
| 10 | 1 | | Flew. |
| 11 | 14 | | No response observed. |
| 12 | 1 | | Moved its wing up and down. |

## TABLE VIII.

RESPONSES OF *Catocala relicta* var. *luctuosa* TO SOUND.

| Number of the Experiment. | Number of Times the Whistle Was Sounded. | Pitch of the Whistle. | Kind of Response. |
|---|---|---|---|
| 1 | 1 | $e^5$ | Made quivering movements with its wings. |
| 2 | 1 | | Ditto. |
| 3 | 1 | | Ditto. |
| 4 | 1 | | Ditto. |
| 5 | 1 | | Ditto. |
| 6 | 1 | | Ditto. |
| 7 | 1 | | Ditto. |
| 8 | 1 | $a^4$ | Flew (It saw the whistle). |
| 9 | 1 | | Whole body quivered. |
| 10 | 1 | | Ditto |
| 11 | 1 | | Ditto. |
| 12 | 1 | | Ditto. |
| 13 | 1 | | Ditto. |
| 14 | 1 | | Ditto. |
| 15 | 1 | | Ditto. |
| 16 | 1 | | Ditto. |
| 17 | 1 | | Ditto. |
| 18 | 1 | | Ditto. |
| 19 | 1 | | Ditto. |
| 20 | 1 | | Ditto. |
| 21 | 1 | | Ditto. |
| 22 | 1 | | Ditto. |
| 23 | 1 | | Moved antennæ gradually forward and then flew. |
| 24 | 1 | | Body quivered. |
| 25–35 | | | Ditto. |
| 36 | 1 | | Flew. |
| 37 | 1 | | Whole body quivered. |
| 38–43 | 1 | | Ditto. |
| 44 | 1 | | Moved its antennæ four times and then flew. |

## TABLE IX.

RESPONSES OF *Catocala robinsoni* TO SOUND.

| Number of the Experiment. | Number of Times the Whistle Was Sounded. | Pitch of the Whistle. | Kind of Response. |
|---|---|---|---|
| 1 | 1 | | Flew. |
| 2 | 1 | | Flew. |
| 3 | 1 | | Flew. |
| 4 | 1 | | Flew. |

## TABLE X.

RESPONSES OF *Catocala vidua* TO SOUND.

| Number of the Experiment. | Number of Times the Whistle Was Sounded. | Pitch of the Whistle. | Kind of Response. |
|---|---|---|---|
| 1 | 1 | $a^4$ | No response observed. |
| 2 | 1 | | Flew. |
| 3 | 1 | | Flew. |
| 4 | 1 | | Flew. |
| 5 | 1 | | Flew. |
| 6 | 1 | | No response observed. |
| 7 | 1 | | Ditto. |
| 8 | 1 | | Ditto. |
| 9 | 1 | | Ditto. |
| 10 | 1 | | Ditto. |
| 11 | 1 | | Ditto. |
| 12 | 1 | | Flew (It saw the whistle). |
| 13 | 1 | | No response observed. |
| 14 | 1 | | Flew. |
| 15 | 1 | | Flew. |
| 16 | 1 | | No response observed. |
| 17 | 1 | | Flew. |
| 18 | 1 | | No response observed. |
| 19 | 1 | | Flew. |
| 20 | 4 | | Flew. |

## TABLE XI.

RESPONSES OF *Catocala vidua*, SPECIMEN NUMBER 2, TO SOUND.

| Number of the Experiment | Number of Times the Whistle Was Sounded. | Pitch of the Whistle. | Kind of Response |
|---|---|---|---|
| 1 | 1 | $a^4$ | Moved its antennæ. |
| 2 | 1 | | Flew. |
| 3 | 1 | | Flew. |
| 4 | 19 | | Flew, but not until after the nineteenth whistle. |
| 5 | 1 | | Flew. |
| 6 | 4 | | No response noticed. |
| 7 | 4 | | No response noted. |
| 8 | 1 | | Flew (Saw the whistle). |

In June, 1914, we made an attempt to see if, in the field, moths could be trained to respond to sounds to which they do not normally respond. We knew that this can be done in the laboratory. Our experience the year before had informed us that most Catocalæ do not respond to sounds of a low pitch. We selected an organ pipe giving 256 vibrations per second. This was sounded several times and if the moth did not respond it was sounded again and simultaneously one of us touched the moth with a brush. We then followed the moth to its next resting place and sounded it again, and if necessary, repeated it over and over.

For these experiments we used; *C. amica*, *C. epione*, *C. neogamma*, *C. ilia*, and *C. innubens*. With *innubens* and *epione* all results were negative. We found two specimens of *ilia* which responded to the sound of the pipe before they had been touched in any manner and one that did not so respond. This response from *ilia* was unexpected, but it militated against using it for these experiments. We succeeded in inducing one specimen of *amica* to respond to the pitch; but failed completely with two others. We experimented with five specimens of *C. neogama*, all males. We induced three individuals of *neogama* to respond to the sound of the organ pipe; but failed with two others. Although the cases in which we succeeded in inducing the moths to respond to sounds to which they do not usually react are few, the fact that we did succeed in a few cases supports our contention that these insects respond only to sounds that have a life significance.

## Conclusions.

1. Our field experiments demonstrate that several different species of *Catocala* moths respond to certain high pitched notes of the Galton whistle; but that they usually do not respond to sounds of low pitch, such as the rumbling of trains, etc.

2. Most specimens responded to those high notes by flying to a nearby tree; but some, and this was especially true of *Catocala relicta*, responded by making quivering movements with its wings.

3. The degree of responsiveness was not the same in all species. Among the least responsive were *C. vidua* and *C. neogama;* at the other extreme were *C. flebilis*, *C. habilis*, and *C. robinsoni*.

4. We do not consider the failure of these moths to respond to certain sounds of low pitch a proof that they do not hear such sounds; indeed, we are inclined to believe that these creatures respond only to such sounds as have a life significance. Three things render this last assumption probable: (1) The fact that *C. unijuga*, which at first did not respond to whistling, did so readily after once a blast of air had been allowed to strike her body simultaneously with the sounding of the whistle; (2) that most of the natural enemies of these moths produce high pitched sounds and trains, and brass bands and other producers of low pitched or coarse sounds do not directly affect the survival of these moths; and (3) by carefully conducted field experiments, we were able to induce three specimens of *C. neogama* to respond to sounds to which the species does not usually react.

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The following list is not intended to be a complete bibliography of the sense of hearing of insects. It contains only such articles as have been referred to in the body of this paper. An effort has been made, however, to include all papers treating of experiments upon the auditory powers of the Lepidoptera. If any such have been omitted, it is because they have escaped our notice.

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# BIOLOGICAL BULLETIN

## SPERMATOGENESIS OF THE HORSE WITH SPECIAL REFERENCE TO THE ACCESSORY CHROMOSOME AND THE CHROMATOID BODY.

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CONTENTS.



### I. INTRODUCTION.

Many interesting things were observed in this study on the spermatogenesis of the horse, but the two points of especial interest and importance are; firstly, the occurrence of a large accessory chromosome, and secondly, the presence of a much smaller though very conspicuous body comparable to the chromatoid body as described by Professor E. B. Wilson ('13) in *Pentatoma*. While the significance of the chromatoid body is problematical, it is a body of extreme interest in this connection on account of its deceptive resemblance to an accessory chromosome. Were it not for the fact that its entire history can be

followed out it might lead to serious misinterpretations. Since the occurrence of the chromatoid body in the horse is so constant and its behavior so distinct, and furthermore, since this is the first case among the vertebrates where such a body has been studied in full detail, it is dealt with at some length in this paper.

The significance of the accessory chromosome is of course obvious. It was shown beyond doubt that sex in the pig is determined by such elements (Wodsedalek,' 13). And while embryological material of the horse is not at present available to enable a similar extended study, the presence and unquestionable behavior of the accessory chromosome giving rise to a dimorphic condition among the spermatozoa of this mammal, nevertheless, lend additional support to the chromosome theory of sex determination. The spermatogenesis of the horse resembles to a certain extent the spermatogenesis of the pig (Wodsedalek, '13), and for the purpose of avoiding too much repetition it is treated in a comparative way in the present study.

This investigation was started in the zoölogical laboratory at the University of Idaho, but the main bulk of the work was done at the Wisconsin Biological Station at Madison. And I wish to thank the zoölogy department of the University of Wisconsin for the liberal use of their laboratories, apparatus and material, and the many other courtesies extended me during the summer of 1914.

## II. Material and Methods.

The material studied, mainly, was obtained from a horse about a year and a half old. Immediately after the testes were removed from the live animal, small pieces were placed in Bouin's and Gilson's fluids. Sections from various parts of the testes were made from four to ten microns thick, and the material fixed in Bouin's fluid and stained with Heidenhain's iron hematoxylin with acid fuchsin as a counterstain, as in the case of the pig, proved to be the most satisfactory.

Material from an animal about a year old was also studied; but while all the stages including the mature sperm could easily be identified in this material, the chromosomes were very difficult to count on account of being too closely aggregated or lumped

together. The finer details of the cells, too, were not as easily made out as in the other material, this being undoubtedly due to the fact that the material was not fixed until about an hour after it was removed from the animal. The chromatoid body, however, was very distinct and could be traced throughout its entire history the same as in the more favorable material. The accessory chromosome, too, could easily be identified, especially through the first spermatocyte division.

## III. General Arrangement of the Germ Cells.

The structure of the testes of the horse differs from the pig in that the seminiferous tubules as well as the corresponding cells in the various degrees of development are much smaller and the interstitial cells are much fewer in number. The continuous network of connective tissue walls is present, but the chambers formed by this network and filled with coiled tubules are much larger in the horse and, therefore, a section through one of the chambers as a rule reveals many more sections of the tubules. These chambers in the horse testes do not show the same regularity in size as is the case in the pig testes. In some cases a group of over a hundred sections through the tubules are surrounded by the connective tissue wall and then again a count of only a dozen or so can be made. The arrangement of the cells in the tubules is similar to that of the other well-known mammals, particularly the pig.

## IV. Spermatogonia.

As a rule the spermatogonia lie in a single layer next to the wall of the tubule, though occasionally some of the cells are crowded out. At times the cells are far apart, in which case they are flattened out on the tubule wall. The cells also differ considerably in size and appearance, depending on the stage of development they are in (Figs. 1–3).

During the resting stages a large nucleolus is invariably present. As a rule it assumes a somewhat heart-shaped appearance; especially is this true in the larger cells and in those in which the chromosomes are beginning to form. A much smaller spherical nucleolus also appears to be fairly constant (Figs. 1

and 2). Other nucleoli varying considerably in size, shape, and number also appear in some of the cells (Fig. 2).

Before the chromosomes begin to form the cells increase greatly in size (Fig. 2). At the conclusion of the resting stage numerous large chromatin granules appear which arrange themselves along fine threads in an entangled mass. The chromosomes soon become distinct and while, as a rule, a count is impossible on account of the overlapping and massing together of the chromosomes, [the mitotic stages were abundant and many distinct counts could be made. Thirty-seven chromosomes appear in the late prophases of the spermatogonial division (Figs. 4 and 5). Thirty-six of these are variously shaped, mainly oblong, and differ somewhat in size. One which is much larger is, as a rule, somewhat triangular or heart-shaped. This is the accessory chromosome and is the same thing as the large nucleolus which appears in the resting stages. That is certain, as the body can easily be traced through the various stages of growth. This condition is similar to that found by Guyer ('10) in man, and Wodsedalek ('13) in the pig. Ordinarily about two thirds of the chromosomes arrange themselves in a ring which encircles the remaining one third. The accessory chromosome may be found anywhere within the mass, and occasionally occurs outside of the main ring, but never far removed from the other chromosomes. During division each chromosome divides in two. The accessory as a rule divides a little in advance of the other chromosomes (Figs. 6 and 7).

The spermatogonia in this, as well as other stages, vary somewhat in size (Figs. 6 and 7). In the smaller cells the cytoplasm appears denser and the chromosomes are more crowded together.

## V. Primary Spermatocytes.

### 1. *Resting Stage.*

The primary spermatocytes arising from the final spermatogonial division in the early resting stage are usually smaller than the spermatogonia immediately preceding and during the division stages. After the disintegration of the chromosomes the nucleus appears much clearer than it does in the later growing stages. The large nucleolus is again very conspicuous and easy

to distinguish from other nuclear bodies when such are present (Figs. 8–11). The small spherical nucleolus again appears to be fairly constant, though at times it is difficult to distinguish it from the other bodies.

### 2. *Synizesis and Growth Period.*

After a brief period of rest the cells begin to increase in size. For some time the nucleus appears much the same as it does in the resting stage of the spermatogonia (Fig. 8). Later it becomes more granular and the linin fibers become more distinct (Fig. 9). Soon after, the chromatin threads become massed in the center of the nucleus (Fig. 10), and later the nuclear wall expands and the entire mass passes to one side of the nucleus, leaving a large clear area in the remaining portion (Fig. 11). This condition is much the same as in the pig except that in that animal the nucleoli were invariably found within the mass of threads and in a position nearest to the nuclear wall, while in the horse the nucleoli are almost invariably within, or next to the clear area (Fig. 11). The nuclear wall in this stage is often very irregular, especially next to the clear portion of the nucleus.

Shortly after the collapse of the chromatin material, the threads pair and appear in about half the original number and twice as thick (Figs. 10–12). There is considerable evidence that pairing of the threads takes place by parasynapsis, and nothing was observed which would indicate that it takes place otherwise; but this phase of the problem demands more study and no positive statement can be made in regard to it at this time. The entire mass of threads then moves toward the center and the large clear area disappears (Fig. 12). The large nucleolus passes toward the periphery of the nuclear wall and the threads soon become evenly distributed. Then follows the period of growth during which time both the nucleus and cytoplasm increase greatly in size (Figs. 13 and 14). The chromatin threads and the large nucleolus also increase considerably in size. It is between the synaptic stage and the fully developed spireme stage that the chromatoid body makes its appearance (Figs. 12–14).

### 3. *Reduction Division.*

Nineteen chromosomes appear in the late prophase or early metaphase stages of the primary spermatocyte (Figs. 15–18). Eighteen of these are the ordinary chromosomes or autosomes and the other is the accessory chromosome. The accessory in this case is practically always found outside of the main mass of chromosomes, either in close contact with them (Figs. 16 and 18), or a short distance away (Figs. 15 and 17). The large size of the eighteen autosomes which are about four times the size of the chromosomes in the spermatogonia indicates that they were formed by the growth and pairing of the thirty-six autosomes found in those cells, while the accessory remains unpaired, making a total of nineteen.

In these cells as in the case of the spermatogonia the chromosomes are frequently bunched together, making an accurate count difficult and often impossible. However, mitotic stages particularly of the first and second spermatocyte divisions were very numerous and among the thousands of cells in mitosis examined several hundred definite counts were made. Figs. 19–29 show the accessory in characteristic positions in the metaphases of division of the primary spermatocyte. The heart-shaped body always passes toward one pole in advance of the other chromosomes and frequently may be found at the pole before the other chromosomes have divided (Figs. 28 and 29). The chromatoid body which is spherical in shape and much smaller than the accessory is also invariably present and very conspicuous. As a rule it is in the spindle, and in a large majority of the cases goes in the direction opposite from the accessory (Figs. 21, 22, 23, 24, 25, 27 and 28), though this behavior is by no means constant, for occasionally it is found with the accessory on the same side of the equatorial plate (Figs. 20, 26 and 29).

When the large, apparently quadrivalent chromosomes divide, the resulting chromosomes are somewhat larger than the chromosomes of the spermatogonia. Immediately after the chromosomes divide they unite in twos (Fig. 29) so that at the time of their arrival at the poles they do not number eighteen, but only nine or exactly one half that number (Figs. 30–35). Additional proof that such a second pairing of the chromosomes occurs lies

in the fact that the resulting nine chromosomes are not one half the size of the original eighteen chromosomes of these cells, but exactly of the same size and apparently quadrivalent. This quadrivalent nature becomes obviated after the division of the secondary spermatocyte, where the resulting chromosomes are bivalent. The primary spermtocyte division is undoubtedly the reduction division and, speaking in terms of univalence, one of the resulting secondary spermatocytes receives eighteen chromosomes and the other eighteen plus the accessory. In terms of bivalence the one type of secondary spermatocytes receive nine chromosomes and the other nine plus the accessory (Figs. 30–33).

## VI. Secondary Spermatocyte.

No resting stage occurs in the secondary spermatocyte, a condition similar to that frequently found in the spermatogenesis of the pig. The second pairing of the chromosomes also takes place here as it does in the pig (Wodsedalek, '13), man (Guyer, '10), and opossum (Jordan, '11). In the pig, however, this pairing takes place much later, never before the cell is completely divided. The secondary spermatocytes divide soon after they are formed and not infrequently the spindles are formed in the two cells resulting from the first spermatocyte division while they are still in close contact. Nine chromosomes arrange themselves in the equatorial plate for division in the one type of secondary spermatocyte (Figs. 42–45), and nine plus the accessory in the other (Figs. 34–38). All of the chromosomes, including the accessory when it is present, divide in these cells (Figs. 36–47). The accessory usually lies a little to one side of the other chromosomes (Figs. 34 and 35), and again, as in the spermatogonia, divides a little in advance of the other chromosomes (Figs. 36–38). This may be due to the partial separation of the two halves of this body even long before the other chromosomes line up for division in this stage (Figs. 21–33). The heart-shape it assumes during the later stages of the primary spermatocyte division and retains during the secondary spermatocyte, is no doubt due to a partial separation at one end of the two components. The chromatoid body remains very conspicuous (Figs. 35–55).

## VII. Spermatids.

The division of the secondary spermatocytes gives rise in the one case to spermatids containing nine chromosomes (Figs. 46, 55 and 56), and in the other case nine plus the one accessory or ten chromosomes (Figs. 39–41). All of the chromosomes except the accessory are bivalent in nature (Figs. 23–41, 54–56), so that in reality we have the equivalent of eighteen chromosomes in the one kind of spermatid and eighteen plus the accessory in the other. All of the foregoing evidences indicate that eighteen is the reduced number of chromosomes.

The accessory is usually out of the main mass of chromosomes (Figs. 40 and 41). Soon after the secondary spermatocyte divides the chromosomes become massed together and the nuclear wall begins to form (Figs. 57–59). In the resting stage half the spermatids contain a large nucleolus which is the same thing as the accessory chromosome, since it can be traced through all the stages in the formation of the nucleus (Figs. 63–65). The other half of these cells lack such a body (Fig. 62). In some cases this nucleolus persists in the developing stages of the spermatozoön (Figs. 72 and 73). Especially is this true in material which has not been destained too much. In favorably stained material the centrosome surrounded by a clear layer can be seen within the centrosphere (Figs. 64–66). The chromatoid body is still very distinct (Figs. 55–67).

## VIII. Development of the Spermatozoa.

The development of the spermatozoön in the horse is essentially the same as the development of the spermatozoön in the pig (Wodsedalek, '13). The centrosome surrounded by a clear area emerges from the sphere (Fig. 67) and soon divides into two spherical bodies (Fig. 68). The anterior one comes in contact with the nuclear wall, while the posterior one which remains spherical passes down the developing axial filament (Figs. 69, 70, 71, 73, 74). This posterior body which is quite small never assumes the shape of a ring as it does in the pig. It passes far down the filament and often no trace of it is left (Fig. 79). Then again it retains a size just enabling detection (Fig. 73). As a rule, however, a sufficient amount of it is left to be sloughed off

as in the case of the pig (Figs. 74, 75, 77 and 84). The chromatoid body is, in rare cases however, also seen on the filament and in such cases apparently fused with the posterior centrosome (Figs. 71 and 76). It is invariably sloughed off before the spermatozoön is fully developed (Figs. 77 and 86).

Shortly after the centrosome divides the nucleus begins to elongate and at the same time migrates toward one end of the cell, so that soon practically all of the cytoplasm is found at the posterior end of the developing sperm (Figs. 67–75). As the acrosome-end of the nucleus comes in contact with the cell-wall no break in the latter is ever noticeable, and the apparent backward pull exerted by the mass of cytoplasm causes the cell-wall to become closely applied to the nuclear wall where it undoubtedly persists as an additional covering of the sperm-head (Figs. 73–79). This supposition that the cell-wall forms an additional covering of the sperm-head is based on two observations; firstly, there is no evidence that the head penetrates the cell-wall, and secondly, the covering of the sperm-head is much thicker after the entire mass of cytoplasm lies at its posterior end (Figs. 73–79). This fact gives one the impression that the distinctly noticeable change in the thickness of the head covering is brought about by the fusion of the two walls. It is also obvious that the cell-wall is not entirely consumed in forming the external covering of the head of the sperm, for it can always be seen surrounding the anterior portion of the axial filament and extending far down into the mass of cytoplasm which is apparently squeezed out of it and about to be thrown off (Figs. 77–79). In the final stages it becomes closely applied to the axial filament and one may safely conclude that the axial envelope is at least partly formed by the portion of the cell-wall extending down from the head (Figs. 77, 79 and 85). This same condition was found to exist in the pig.

When the developing sperms reach the stage represented in Fig. 73 they become attached in clusters to the large nurse cells. As the sperms develop the cytoplasmic mass of the nurse cells decreases. Just as the mass of cytoplasm is being thrown off by the developing spermatozoa, the latter leave the nurse cells and become embedded in the layer of cytoplasm composed of the

cast-off masses, apparently nursing on the material so that little of it, if any, goes to waste.

Every stage in the sloughing off of the cytoplasmic mass can easily be observed (Figs. 77–86). When these masses of naked cytoplasm are completely sloughed off they assume a rounded shape and if the chromatoid body is present they might, at first sight, be mistaken for minute cells with the chromatoid body as the nucleus. And I feel that Wilson ('13), in speaking of this condition in *Pentatoma*, is absolutely correct when he says, "I also think it probable that the bodies that have been described as 'degenerating cells' in the late spermatid-cysts by some observers are identical with the protoplasmic balls here described."

Among the cast-off balls four different types can be observed (Figs. 80–83). One type contains a small body which apparently is the remnant of the posterior centrosome (Fig. 80); another type is clear and one is led to believe that in such a case the centrosome was entirely consumed (Fig. 81); another shows the same condition regarding the small body but contains the chromatoid body (Fig. 86); and still another contains both the chromatoid body and the much smaller centrosome remnant (Fig. 82). Later when the spermatozoa are fully developed the roundish masses become irregular in shape and finally begin to disintegrate. The disintegration is characterized by the breaking-up of the masses into small particles and by the appearance of many deeply staining bodies and globules which vary considerably in size (Fig. 83).

Occasionally in the last stages of the disintegration of the cytoplasmic material and also when the material entirely disappears there may be seen small, deeply staining bodies identical in size and appearance to the chromatoid body and one is led to believe that it is the same thing (Figs. 83 and 84). If it is the same thing the fact throws some light on its durable consistency.

The mature spermatozoön in general resembles that of the pig, except that it is smaller, and the head is thinner at the anterior end and thicker at the posterior end. The entire nucleus enters into the formation of the head and the contents become homogeneous and intensely staining.

## IX. Variation in Size of Adult Spermatozoa.

The spermatozoa of the horse like those of the pig vary considerably in size and many careful measurements show that they, too, are of two distinct types, the one being much larger than the other. Mature specimens which were free in the lumen of the tubule and parallel to the objective, were selected at random and outline sketches of six hundred heads enlarged (× 2,000) were made with the aid of a camera lucida. The lengths of the sketches were then measured and recorded in quarter millimeters. It can be seen from Fig. 1 in the text that two separate types of

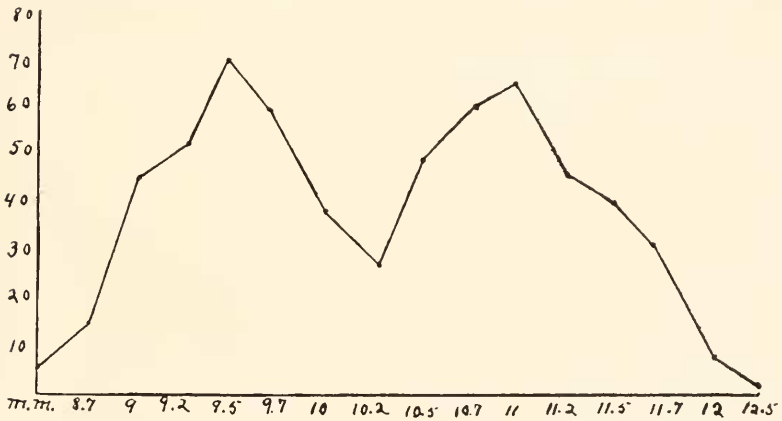


Fig. 1. Diagram showing the variation in size among six hundred mature horse spermatozoa. Figures at the left give the numbers of individuals belonging to each type. Figures at the bottom give the lengths of the heads of the spermatozoa in millimeters, magnified two thousand times.

spermatozoa exist; the greatest number of the one kind measuring 9.5 mm., and of the other 11 mm. I think it is safe to assume that this dimorphic condition in the size of the mature spermatozoa is due to the accessory chromosome. The increased size in the one type is due presumably to the presence of that element.

A similar dimorphic condition was found to exist among the spermatozoa of the pig; one type measuring from 11 to 12 mm., and the other from 14 to 14.5 mm. (Wodsedalek, '13). Size dimorphism also exists in the adult spermatozoa of *Anasa tristis* (Faust, '13). This of course is exactly what would be expected

since the spermatogenesis studies of this form indicate that one half of the spermatozoa receive one more chromosome each than the other half (Paulmier, '99).

## X. Migration of the Developing Spermatozoa.

In the very beginning of the transformation of the spermatids into spermatozoa when the acrosome takes a position on the nucleus opposite the dividing centrosome (Fig. 68), the anterior end of the sperm-cell which bears the acrosome, invariably points in the direction of the tubule wall and the Sertoli or nurse cells. Long before the tail is sufficiently developed to aid in locomotion these sperm-cells move a short distance and come in contact in bunches with the nurse cells. There apparently exists some attraction between the nurse cells and the nuclei of the sperm cells in that stage of development. In this first stage of migration only the nucleus appears to be attracted while the cytoplasm exhibits a tendency to remain in place. The fact that the cytoplasm does have a tendency to remain in place while the nucleus or sperm-head moves forward undoubtedly accounts for the posterior position that the entire mass of cytoplasm assumes with respect to the sperm-head (Figs. 69–79). This change in position of the cytoplasm to the posterior end of the developing cell occurs simultaneously with the migration of the cell, which is further evidence for such an assumption. The nucleus is apparently attracted with sufficient intensity to enable it to pull the entire cytoplasmic contents after it to a certain extent. In the later stages the movement of the sperm-head deeper into the cytoplasm of the nurse-cell is probably facilitated by the flagellum-like motion of the filament which extends a considerable distance out of the cell (Figs. 75–79). Later, when the spermatozoa are almost fully developed and slough off the balls of naked cytoplasm (Figs. 85 and 86) they back out away from the nurse-cells, becoming embedded in the cast-off material where they remain scattered until they are fully developed and then become free in the lumen of the tubule. This indicates that the sperms in that stage of development are attracted more by their own thrown-off material than by the rather scanty contents of the nurse cells, which are then very

much collapsed, owing to the large number of developing sperms which they have nourished. This migration of the sperm backward is probably nothing more than a chemotactic response to the food contained in the balls of cytoplasm.

## XI. The Chromatoid Body.

The behavior of the chromatoid body in the horse bears a striking resemblance to the behavior of the chromatoid body described in *Pentatoma* (Wilson, '13). Dr. Wilson treats the subject at considerable length in his paper and therefore much of the detail concerning this body in the horse may safely be omitted. However, all the more essential features will be presented here since this is the first case among the vertebrates, according to my knowledge, where such an element has been studied in full detail. The reader is advised to familiarize himself with Professor Wilson's article in order to appreciate fully the surprising similarity existing in the behavior of the chromatoid body in such diverse classes of animals as the insects and the mammals.

In speaking of the chromatoid body Professor Wilson ('13) says in part, "As seen during the growth-period and the spermatocyte-divisions it is of rounded form, dense and homogeneous consistency, and after double staining with hæmatoxylin or safranin and light green is at every stage colored intensely blue-black or brilliant red, precisely like the chromosomes of the division-period or the chromosome-nucleoli of the growth-period. In the first spermatocyte-division it may lie anywhere in the cell, sometimes almost at the periphery, but is often close beside the chromosomes. In the latter case it usually lies in, on or near the spindle, lags behind the chromosomes during the anaphases, and in later stages is found near one pole, presenting an appearance remarkably like that of an accessory chromosome (Figs. 8–10). For such in fact I mistook it, even after the discovery that a similar body is often also seen near one pole in the *second* division (Figs. 22, 23); for I supposed this might be a case like that of *Ascaris megalocephala*, where, according to Edwards ('10) the *X*-chromosome may pass undivided to one pole in either the first or second division. The resemblance is indeed most deceptive; and these division-figures have often been exhibited to

other observers as "a remarkably clear demonstration of an accessory chromosome" without at first arousing the least suspicion of the hoax.

"The body in question is nevertheless neither an accessory nor any other kind of chromosome; though this did not become wholly certain until after a study of the entire spermatogenesis. It is in fact of protoplasmic origin, first appearing early in the growth-period outside the nucleus, whence it may be followed uninterruptedly through all the succeeding stages until it is finally cast out of the spermatozoön. Upon dissolution of the nuclear membrane it is left lying near the chromosomes, passes without division into one of the daughter-cells in each of the spermatocyte-divisions, and thus enters but one fourth of the spermatids."

In the horse the chromatoid body is of a spherical shape and also of a dense and homogeneous consistency, and stains exactly like the chromosomes of the division stages or the chromosome-nucleoli of the growth-period. It is invariably surrounded by a clear area. It makes its appearance in the stages immediately following synizesis and apparently attains its full size rather abruptly, for as a rule even in the earlier growing stages, if it is present at all, it possesses its full size, although in some cases it was found to be somewhat smaller (Fig. 13). Occasionally, in the earliest stages immediately following synizesis one or two very small bodies within clear vacuoles could be detected (Fig. 12). Two such small bodies are extremely rare and even the single minute bodies showing the very beginning of the chromatoid body are not numerous; however, it is quite certain that the body practically always originates as a single element. When the cells attain their maximum size the chromatoid body is invariably present and possesses its full size which makes it very conspicuous (Fig. 14).

The chromatoid body may be seen anywhere within the cytoplasm, either near the nucleus or far from it. Sometimes it appears to be in fairly close contact with the cell-wall (Fig. 14). When the nuclear wall disappears and the chromosomes come into full view, it may again be found anywhere in the cytoplasm. Later when the chromosomes arrange themselves for division

in the equatorial plate it most generally takes a position near them (Figs. 15 and 18), and when the spindle is formed, in a large majority of the cases, it takes a position in, on or near the spindle (Figs. 21–28) as is the case in Pentatoma (Wilson, '13). This, however, is not always the case, for occasionally it is far away from the spindle (Figs. 20 and 29).

It was in the primary spermatocyte division that the chromatoid body was first observed. It attracted my attention at the very first glance at the material under low power of the microscope and its constant appearance in this stage led me to suppose, at first, that it may be an accessory chromosome. Soon, however, the large, heart-shaped accessory was discovered and for some time I had the impression that this was the *X*-chromosome and the small spherical body the *Y*-chromosome. This temporary, erroneous impression was obtained through the peculiar fact that in about ninety per cent. of the cases the chromatoid body passes over to the half of the dividing cell opposite from that containing the large accessory, and in almost a hundred of the first mitotic stages examined not a single case was noticed in which the spherical body was on the same side of the equatorial plate with the accessory chromosome. Even when the first case in which both of the bodies were seen on their way to the same pole was observed, the matter was not taken very seriously. Later, however, when more such cases were seen, my suspicion was aroused and further observations convinced me that besides the supposed *y*-chromosome a body identical to it was present. And it was not until the entire history of the body could be traced from the growth-period to the casting-off of the mass of cytoplasm in the final stages of the developing spermatozoön, that I was absolutely certain that the suspicious looking element and the supposed *y*-chromosome were one and the same thing, namely, the chromatoid body, first described by Wilson in insects.

In exceptionally rare cases, one (Figs. 23 and 26) or two other small, deeply staining bodies within clear vacuoles occur in the cytoplasm (Fig. 27). However, in cases where such bodies do occur, there is no appreciable difference in the size of the chromatoid body and therefore it is difficult to determine whether such bodies are simply portions split off from the chromatoid

body, or whether they originate separately. In only three cases did I observe two bodies apparently of equal size and smaller than the profoundly constant chromatoid body (Fig. 54). Were such cases more numerous one might assume that such bodies are the components of the chromatoid body, but since such bodies are of such extremely rare occurrence no definite statement can be made in regard to them.

When the primary spermatocyte divides the chromatoid body is practically always found in only one of the resulting cells (Figs. 30–33) and in a large majority of the cases it is found in the cells which do not contain the accessory chromosome (Figs. 30, 31 and 33). This, however, is not universal, for in some cases at least, it is found in the same cell which contains the accessory (Figs. 32 and 35); and it has also been seen in the division stages of such a type of secondary spermatocyte (Figs. 38 and 39) as well as in the spermatid resulting from such a division (Fig. 41). In the anaphase of the secondary spermatocyte division the body is usually seen lagging on the spindle threads behind the masses of chromosomes (Figs. 39, 48, 49, 51 and 52); occasionally, however, it is seen at the pole (Fig. 51). After the division is complete the body usually lies far out in the cytoplasm (Figs. 41 and 57), and in rare cases only, is it seen in close contact with the nucleus. Figure 58 represents an extreme case of that nature, and it appears that such a condition is brought about when the chromatoid body bears a relation to the chromosome as is represented in Fig. 51. Sometimes two bodies (Fig. 60), though not always of the same size, appear in the spermatid.

In the late resting stages of the spermatid the body may again be found anywhere in the cytoplasm (Figs. 62–67), at times near the nucleus (Fig. 62). Sometimes it is found in close contact with the centrosome (Figs. 63 and 66) and in only rare cases it is found on the axial filament, giving the impression that it is fused or in close contact with the posterior centrosome (Figs. 72 and 76). Later, however, it leaves the filament and lies freely in the cytoplasm (Figs. 74, 75, 77, 86). In the final stages of the developing spermatozoön when the cytoplasmic mass is cast off, the chromatoid body when present is invariably thrown off with it (Figs. 82 and 86). It is certain that the chromatoid

body does not contribute in any visible way to the formation of the spermatozoön. The foregoing facts also indicate that great care must be exercised in interpreting the significance of bodies which appear like chromosomes, but really are something entirely different and no positive statements can be made regarding their meaning unless their entire history can be definitely traced.

It is very probable that a body similar to the chromatoid body in the horse also exists in the pig. In speaking of a small chromatin body which frequently occurs in the first spermatocyte division of the pig (Wodsedalek, '13), I make the following statement: "Occasionally a small chromatin body is present in this first spermatocyte division (Figs. 28, 31, 32, 35 and 37). Fig. 31 shows such a body passing to the same pole with the accessories, in advance of the other chromosomes. Fig. 32 represents an earlier stage of much the same thing. In Fig. 35 it can be seen passing to the opposite pole, and Fig. 37 represents an extremely rare case where two such bodies are present, one somewhat larger, passing to either pole, even in advance of the two accessory chromosomes. While the small body can be seen frequently, as a rule no such element can be detected, and while it may possibly be comparable to the small pair of chromosomes found so constantly in some of the Tracheata, my present data on its irregular occurrence and behavior do not permit a conclusion regarding its significance."

Further investigation regarding the body in question in the pig will be taken up presently. It might also be mentioned here that the chromatoid body is present in the germ-cell of the bull. A complete account of its behavior in that animal will be published later.

## XII. Summary.

1. Thirty-seven chromosomes differing somewhat in size occur in the spermatogonia. One, the accessory, is distinctly larger than the others.

2. In the spermatogonial division the accessory divides a little in advance of the other chromosomes.

3. Nineteen chromosomes appear in the primary spermatocyte division, of which eighteen are evidently bivalent and the other is the accessory.

4. In the secondary spermatocyte division the heart-shaped accessory passes undivided to one pole in advance of the other chromosomes.

5. The primary spermatocyte division is evidently the reduction division, giving rise to two different types of secondary spermatocytes; one with the accessory and the other lacking it.

6. There is no resting stage following the first spermatocyte division.

7. A second pairing of the chromosomes takes place so that only one-fourth the original number of chromosomes appear for division in the secondary spermatocyte.

8. The accessory chromosome divides in the secondary spermatocyte division a little in advance of the other chromosomes the same as it does in the spermatogonia.

9. The one type of secondary spermatocyte, which contains the accessory, gives rise to two spermatids, each containing the accessory and nine bivalent chromosomes.

10. The other type of secondary spermatocyte, which lacks the accessory, gives rise to two spermatids, each containing only the nine bivalent chromosomes.

11. In terms of univalence, then, one type of spermatid receives eighteen chromosomes plus the accessory and the other type receives only the eighteen ordinary chromosomes.

12. In view of the foregoing facts, two different types of spermatozoa, equal in numbers, are produced in the horse; the one type contains in addition to the ordinary chromosomes the accessory, and is apparently the female determining spermatozoön.

13. Actual measurements of six hundred mature spermatozoa reveal the interesting fact that two distinct types of spermatozoa as regards size are produced, the one being much larger and presumably the one which bears the accessory chromosome.

14. The dimorphic condition among the spermatozoa of the horse lends additional support to the chromosome theory of sex determination.

15. The developing spermatozoa invariably cast off a mass of cytoplasm.

16. A chromatoid body, which simulates the appearance of a

*y*-element in the primary spermatocyte division stages, makes its appearance during the growth period and can be traced forward until it is finally thrown off with the ball of cytoplasm in the developing spermatozoön. It does not contribute in any visible way to the formation of the spermatozoön.

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## EXPLANATION OF PLATES.

### PLATE I.

(All of the drawings were made with the aid of a camera lucida, × 2,400.)

FIG. 1. Early spermatogonial cell showing a large triangular nucleolus and two small nucleoli, one of which is spherical. Other cells in the same stage often show many more nucleoli.

FIG. 2. Resting stage of a full grown spermatogonial cell showing the large triangular nucleolus and several small nucleoli, one of which is spherical and can frequently be detected.

FIG. 3. Prophase of a spermatogonial division in which the chromosomes are still rather indistinct.

FIGS. 4 AND 5. Late prophase of spermatogonial division showing thirty-six ordinary chromosomes and the large accessory which can easily be distinguished.

FIGS. 6 AND 7. Metaphase of division in a spermatogonium showing the accessory dividing in advance of the other chromosomes. In Fig. 6 the cell appears smaller and the chromosomes are more crowded.

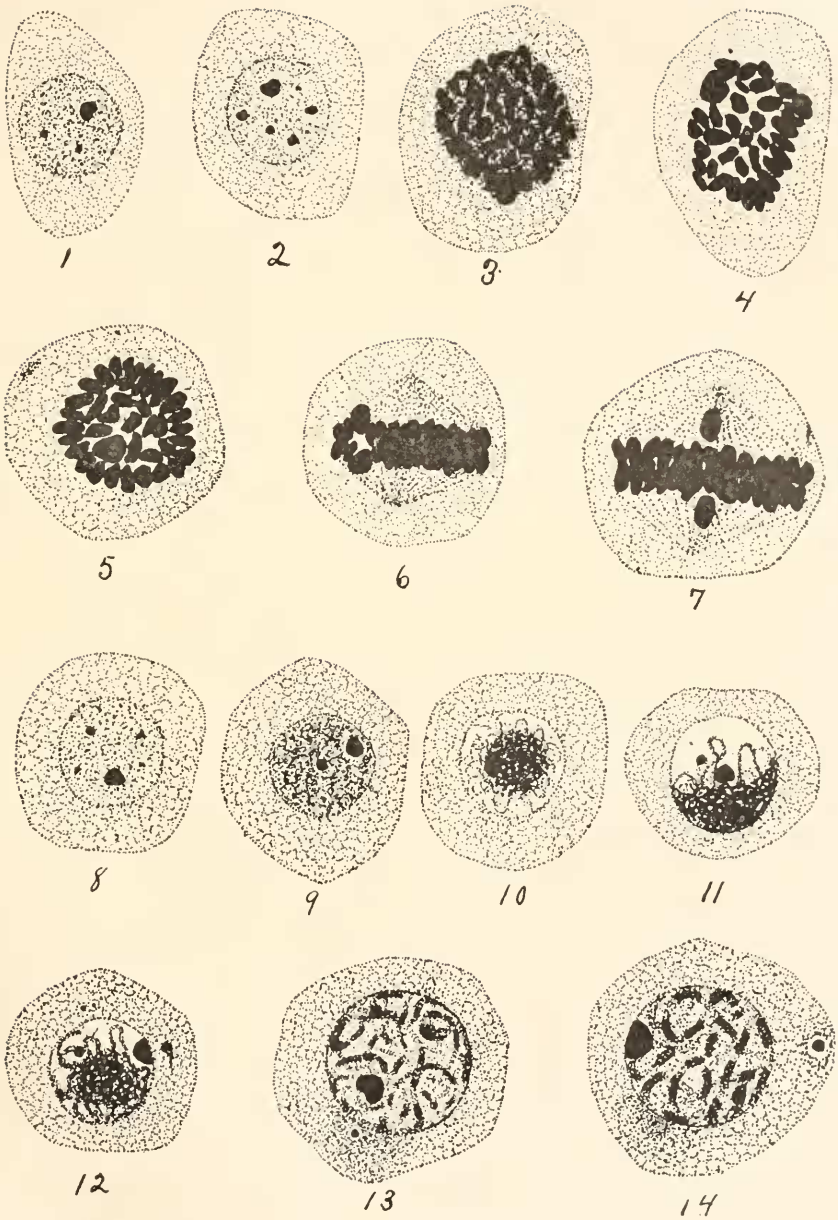
FIGS. 8 AND 9. Early and late resting stages of a primary spermatocyte, respectively. Both show the large and the small nucleolus.

FIG. 10. Primary stage just before synizesis showing a mass of fine threads and the two nucleoli.

FIG. 11. Primary spermatocyte in synizesis showing the nucleoli in a characteristic position out of the mass of threads.

FIG. 12. Primary spermatocyte following synizesis and synapsis. The threads scatter about in the nucleus.

FIGS. 13 AND 14. Spireme stage of a primary spermatocyte showing increase in size of the cytoplasm, nucleus and the large nucleolus, and the beginning of the chromatoid body.

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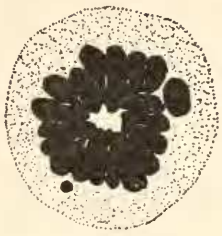
## Plate II.

Figs. 15–18. Late prophases of primary spermatocytes showing eighteen large chromosomes, the accessory a little off to one side and the conspicuous chromatoid body anywhere in the cytoplasm. Fig. 16 shows a characteristic bunch of chromosomes in which a count is impossible.

Figs. 19–26. Metaphase of division in primary spermatocyte, showing the accessory chromosome in characteristic positions passing to the pole, and also the chromatoid body. Figs. 20 and 26 show the chromatoid body with the accessory on the same side of the equatorial plate. Figs. 23 and 26 show also an extra small body.



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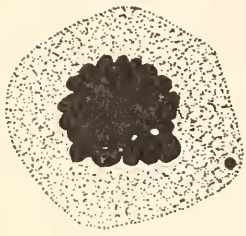

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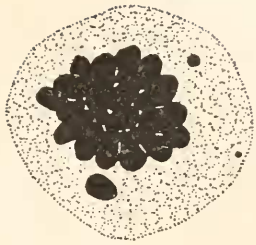

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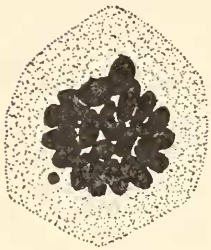

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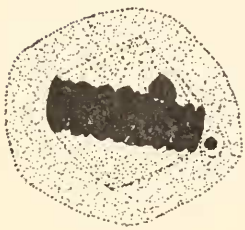

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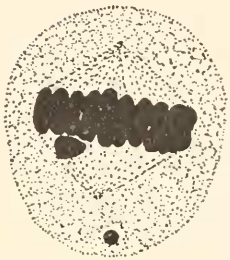

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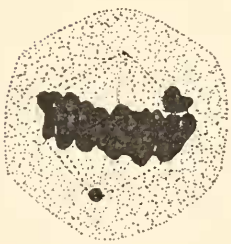

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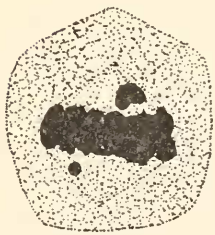

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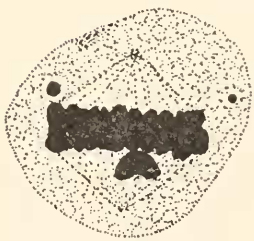

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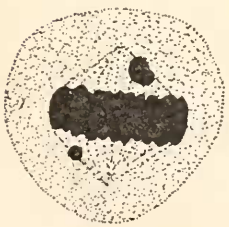

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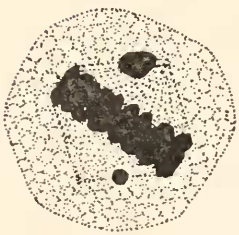

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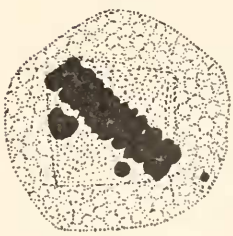

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PLATE III.

FIGS. 27, 28, AND 29. Metaphase of division in primary spermatocyte showing the accessory chromosome and the chromatoid body. Fig. 27 shows two other small and deeply stained bodies. Fig. 28 shows the accessory at one pole and the chromatoid body at the other. Fig. 29 shows the accessory at the pole, and the chromatoid body off the spindle and near the periphery of the cell.

FIGS. 30 and 31. Late anaphase of division in primary spermatocyte showing nine large chromosomes and the accessory at one pole, and nine large chromosomes and the chromatoid body at the other.

FIG. 32. Late anaphase of division in primary spermatocyte, showing nine chromosomes at one pole, and nine chromosomes, the accessory, and the chromatoid body at the other.

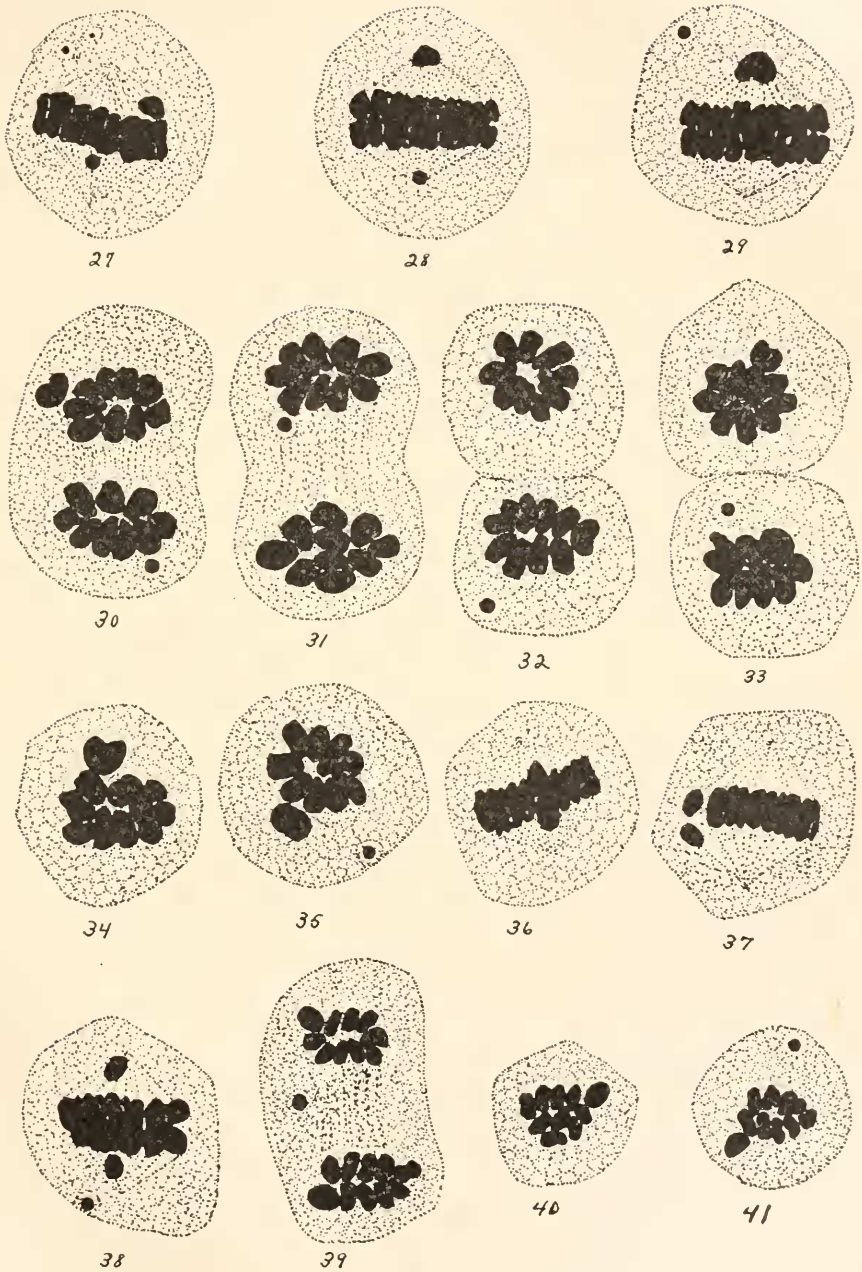
FIG. 33. Two resulting cells of a primary spermatocyte division, one containing the accessory chromosome and the other the chromatoid body.

FIGS. 34 and 35. Late prophase of division in a secondary spermatocyte which received the accessory chromosome. Cell represented in Fig. 35 also shows the chromatoid body.

FIGS. 36, 37, AND 38. Metaphase of division in the secondary spermatocyte showing the division of the accessory in advance of the other chromosomes. Fig. 38 also shows the chromatoid body near the periphery.

FIG. 39. Late anaphase of division in a secondary spermatocyte which received the accessory chromosome, nine apparently bivalent chromosomes and the large accessory can be seen at either pole and the chromatoid body is between the two masses of chromosomes.

FIGS. 40 AND 41. Spermatid showing nine bivalent chromosomes and the accessories. Fig. 41 also shows the chromatoid body.

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## Plate IV.

Figs. 42, 43, and 44. Late prophase of division in a secondary spermatocyte which did not receive the accessory chromosome, showing only the nine ordinary chromosomes. Figs. 42 and 43 show also the chromatoid body.

Figs. 45 and 46. Early metaphase of division in a secondary spermatocyte which did not receive the accessory chromosome. Fig. 45 shows the chromatoid body off the spindle.

Figs. 47–54. Late anaphase of division in secondary spermatocytes showing various positions of the chromatoid body when it is present. Fig. 47 shows also a small body in addition to the chromatoid body. The cell is one which did not receive the accessory chromosome. In the cell represented in Fig. 50 the chromatoid body was absent and in Fig. 54 two bodies may be seen.

Figs. 55 and 56. Spermatid showing nine bivalent chromosomes which is one of the resulting cells of the division of a secondary spermatocyte which did not receive the accessory chromosome. Fig. 55 shows the chromatoid body.

Fig. 57. Characteristic massing of the chromosomes just before the nuclear wall of the spermatid is formed.

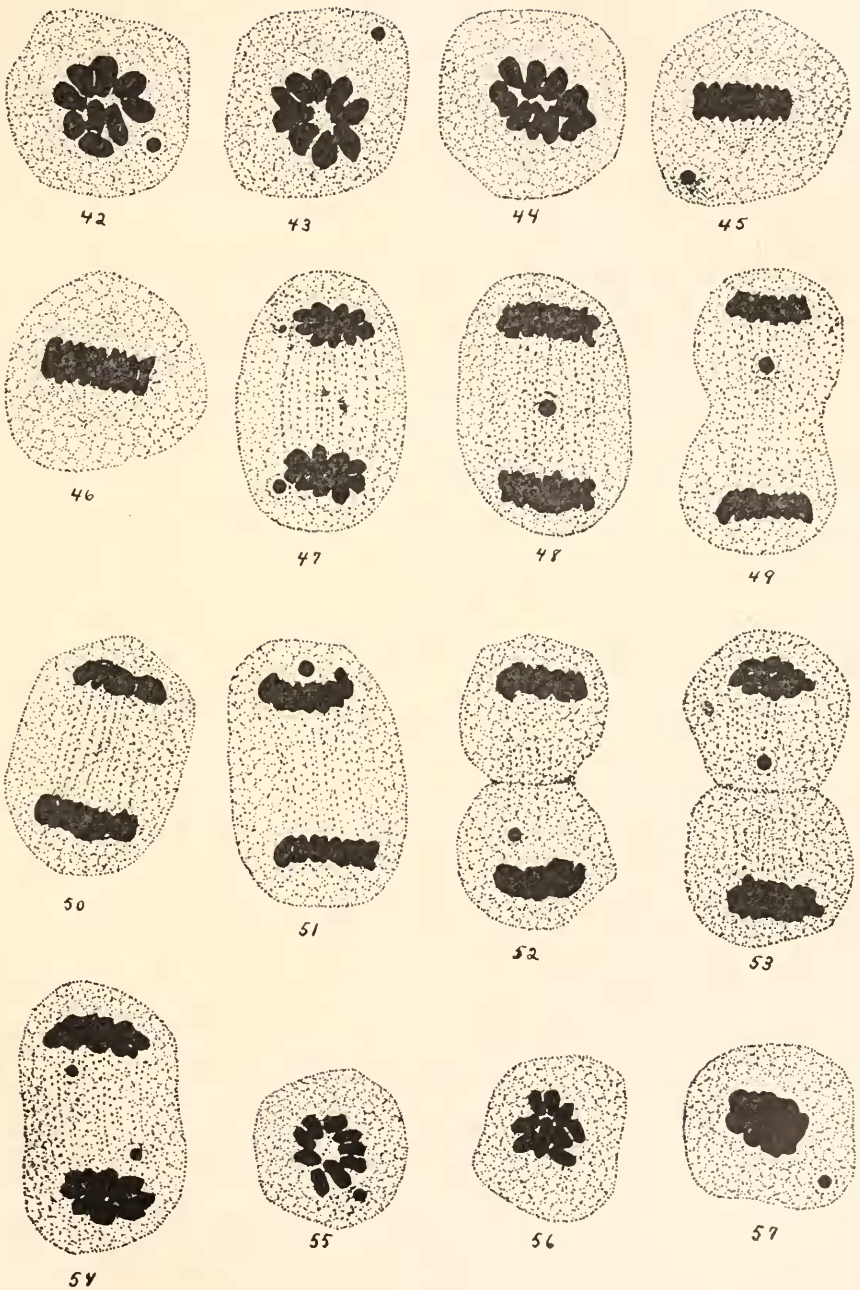


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PLATE V.

FIGS. 58–61. Early spermatids showing the characteristic structure of the nucleus, and the position of the chromatoid body. Fig. 60 shows two bodies of practically the same size as the chromatoid body.

FIGS. 62–67. Resting stage of the spermatid. Figs. 62–65 show the large nucleolus or the accessory chromosome. Fig. 62 shows the chromatoid body near the nucleus; Fig. 63 shows it very near the centrosome which is out of its sphere; Fig. 65 shows it at the periphery of the cell; Fig. 66 shows it near the centrosphere; and Fig. 67 shows it far from the centrosphere out of which the centrosome had just emerged.

FIGS. 68–73. Early stages of the developing spermatozoön. Fig. 68 shows the divided centrosome, the very beginning of the axial filament, and the acrosome which had migrated to the anterior end of the nucleus or sperm-head; Fig. 69 shows the posterior centrosome passing down the axial filament, and the chromatoid body far down in the cytoplasm away from the filament; Fig. 70 shows the same thing except that the chromatoid body is not present; Fig. 71 shows the chromatoid body near the posterior centrosome; Fig. 72 shows what apparently is the fusion of the chromatoid body with the posterior centrosome; and in Fig. 73 the chromatoid body is absent and the posterior centrosome is far down the axial filament and so small that it can scarcely be detected.

FIGS. 74 AND 75. Later stages of the developing spermatozoön showing the chromatoid body in the cytoplasm at the posterior end. Fig. 74 shows the posterior centrosome still on the filament, while Fig. 75 shows that it had been sloughed off.

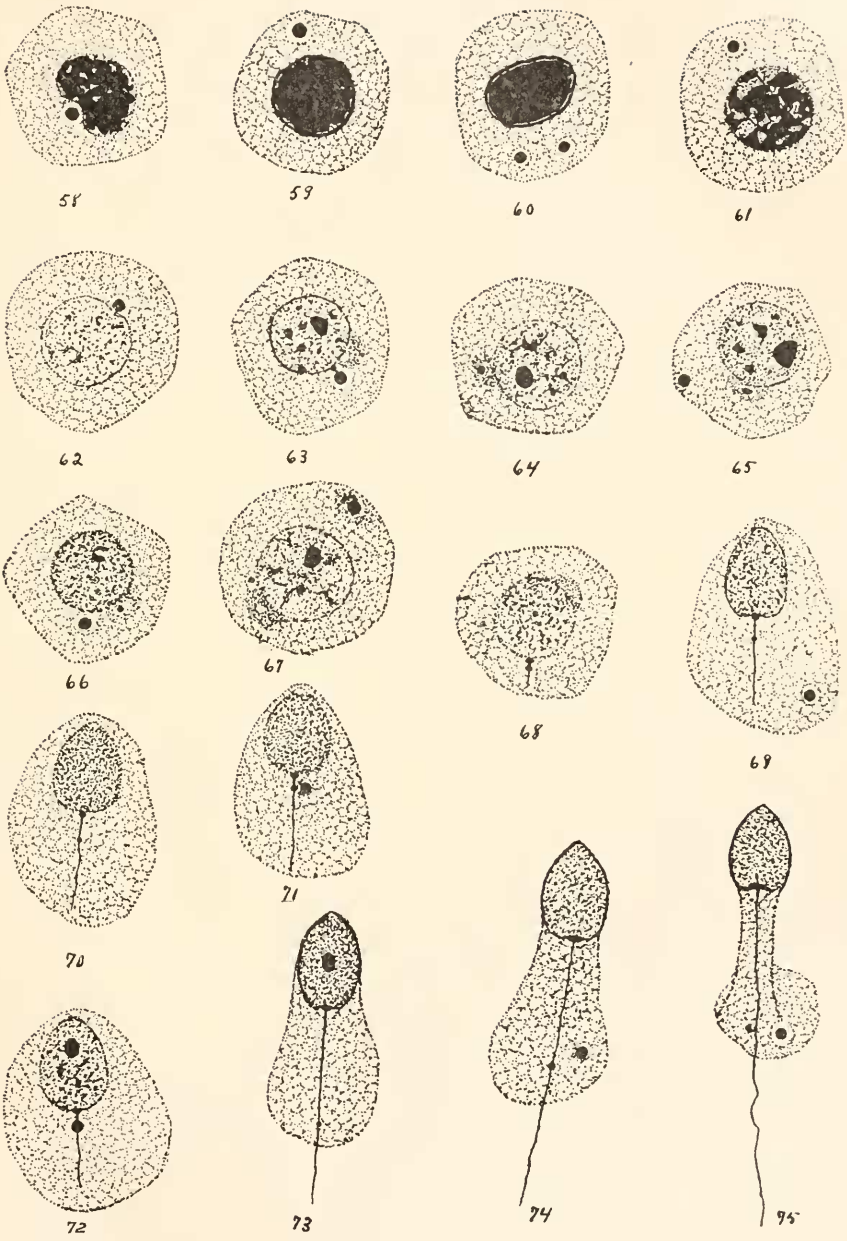


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PLATE VI.

FIGS. 76–79. Later stages of the developing spermatozoön. Fig. 76 shows the chromatoid body far down on the axial filament; Fig. 77 shows the chromatoid body very close to the posterior centrosome; Fig. 78 shows the sloughed-off centrosome, but the chromatoid body is absent; and in Fig. 79 both of these bodies are lacking

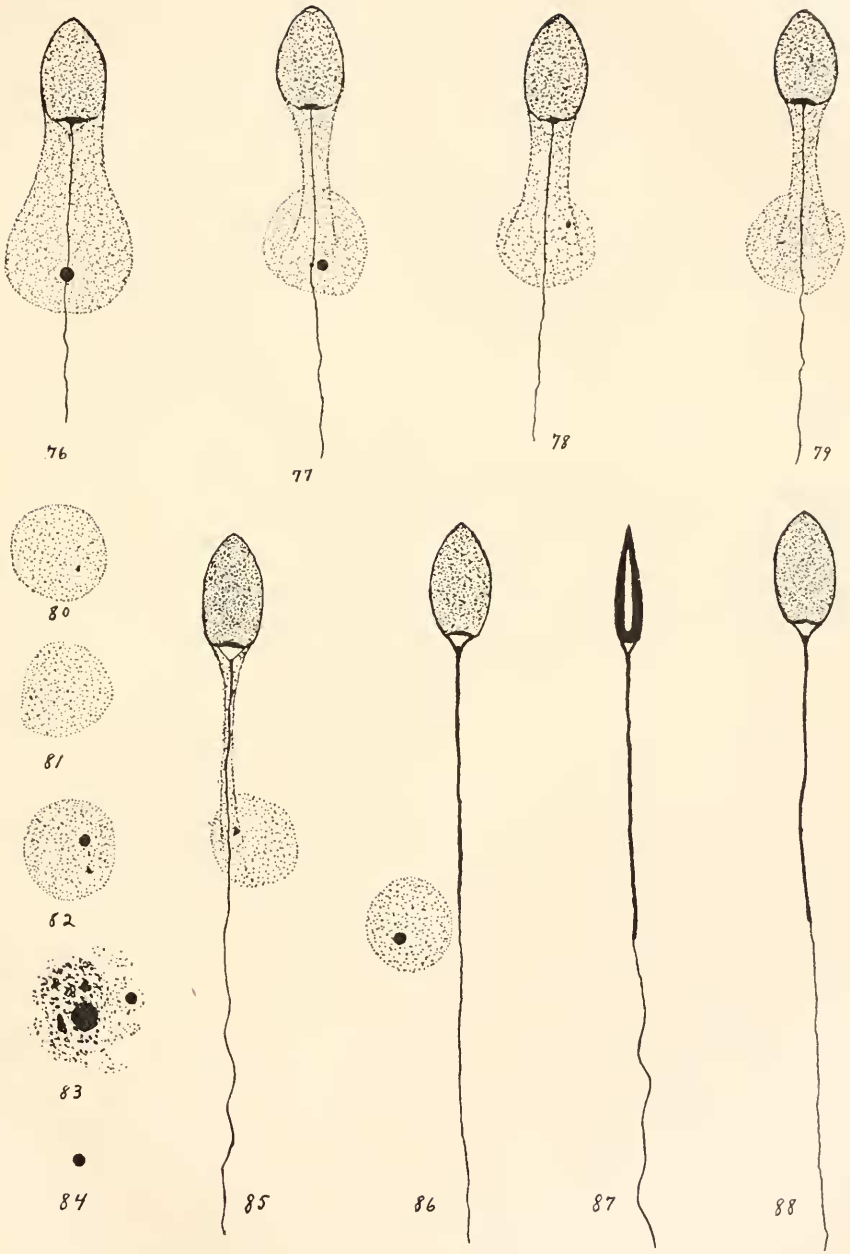
FIGS. 80–83. Cast-off balls of cytoplasm. Fig. 80 shows a small body which apparently is the sloughed-off centrosome; Fig. 81 shows neither the centrosome nor the chromatoid body; Fig. 82 shows both bodies; and Fig. 83 represents the ball of cytoplasm in the process of degeneration.

FIG. 84. A deeply staining body occasionally found in the lumen of the tubule and probably the same thing as the chromatoid body.

FIGS. 85 AND 86. Final stages in the developing spermatozoön. Fig. 85 shows that the cytoplasmic ball is about to be thrown off; and Fig. 86 shows the cytoplasmic mass together with the chromatoid body completely separated from the spermatozoön.

FIG. 87. Side view of a mature spermatozoön.

FIG. 88. A mature spermatozoön.

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# AN EXPERIMENTAL STUDY OF THE AUDITORY POWERS OF THE GIANT SILKWORM MOTHS (SATURNIIDÆ)

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This is a companion paper to the "Auditory Powers of the Catocala Moths" by C. H. Turner and Ernst Schwarz. The latter paper embodies the results of a field study and this epitomizes a laboratory investigation. The habits of resting quietly upon a tree trunk and of flying, when disturbed, to a nearby tree renders the Catocalæ excellent material for field study; the fasting habits of the Saturniidæ render them equally good material for laboratory work. The paper on the Catocala moths contains both a historical resume and a bibliography; hence they are not needed in this contribution.

In these experiments the following moths were used: 79 specimens of *Samia cecropia* Linn., 104 of *Philosamia cynthia* Drury, 41 of *Callosamia promethea* Drury and 81 of *Telea polyphemus* Cramer. These insects were confined beneath wire dish covers. Each moth was numbered and one insect, in case of mated individuals one pair, was placed beneath a cover.

These experiments were conducted in an out-of-doors insectary the north wall of which is constructed almost entirely out of wire netting. The other three wooden walls are window-less and lined with shelves. These walls and the shelf-rests are supported by the ground. The wooden floor rests on the ground, but is not attached either to the walls or the shelf-rests; indeed, a space of from one to three feet separates the floor from the walls. Suspended from the ceiling by picture wire, there is a heavy swinging shelf. The subjects of these experiments were kept on these shelves. Since I always stood on the floor when sounding any of the instruments, it was impossible for the vibrations to reach the moths by any medium other than the air.

These experiments were conducted in the mornings between five and half past seven and in the afternoons between three and seven. On Saturdays and Sundays experiments were sometimes conducted all day long.

For producing stimuli the following instruments were used: an adjustible organ pipe, with a range for all notes of two octaves and for one note of three; an adjustable pitch pipe, and an Edelmann's Galton whistle. Such moths as responded did so by moving the wings as though about to fly. In the early experiments, before I had many moths on hand, each moth was tested with all of these instruments; because I hoped to determine the upper and lower threshold of hearing for each specimen. Later on, partly because I became convinced that there are theoretical

## Table I.

*Number*: 3–VI–14: 1. *Specimen: Callosamia promethea*, female.

*Place:* Confined, under a wire dish cover, on the swinging shelf.

*Method:* At each trial the instrument was sounded five times at intervals of a minute and records made of the moth's behavior.

| Trials. | Date. | Time. | Stimulus. | Vibrations Per Second. | Temperature. | Tests. | | | | | Remarks. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 1 | 2 | 3 | 4 | 5 | |
| 1 | 3–VI | 6:30 | P.P. | 680 | 71 | * | * | * | * | * | Response vigorous. |
| 2 | 4–VI | 6:00 | P.P. | 680 | 78 | * | * | * | * | * | Response vigorous. |
| 3 | 4–VI | 6:10 | G.W. | 3,480 | 78 | — | — | — | — | — | |
| 4 | 4–VI | 6:15 | O.P. | 512 | 78 | * | * | * | * | * | Response vigorous. |
| 5 | 4–VI | 6:20 | O.P. | 256 | 78 | * | * | * | * | * | Response slight. |
| 6 | 4–VI | 6:25 | O.P. | 128 | 78 | * | * | * | * | * | |
| 7 | 4–VI | 6:30 | O.P. | 64 | 78 | * | * | * | * | * | |
| 8 | 6–VI | 10:05 | P.P. | 680 | 86 | — | — | — | — | — | Whistle held in rear. |
| 9 | 6–VI | 10:10 | O.P. | 512 | 86 | — | — | — | — | — | Whistle held in rear. |
| 10 | 6–VI | 10:15 | O.P. | 256 | 86 | — | — | — | — | — | Whistle held in rear. |
| 11 | 6–VI | 10:20 | O.P. | 128 | 86 | — | — | — | — | — | Whistle held in rear. |
| 12 | 6–VI | 10:25 | O.P. | 64 | 86 | — | — | — | — | — | Whistle held in rear. |
| 13 | 6–VI | 10:30 | P.P. | 680 | 86 | * | — | — | — | — | Whistle held in front. |
| 14 | 6–VI | 10:35 | O.P. | 256 | 86 | * | * | * | * | * | Whistle held in front. |
| 15 | 6–VI | 15:00 | P.P. | 680 | 96 | * | * | * | — | — | Whistle held in front. |
| 16 | 6–VI | 15:10 | O.P. | 256 | 96 | — | * | — | * | * | Whistle held in rear. |
| 17 | 6–VI | 15:20 | O.P. | 64 | 96 | — | — | — | — | — | |
| 18 | 6–VI | 15:30 | O.P. | 256 | 96 | * | * | * | * | * | |

Explanation of abbreviations; G.W. means Galton whistle; O.P., organ pipe; P.P., pitch pipe; in the second column, the roman numerals stand for months and the Arabic for days; in the third column, the hours are numbered from 1 to 24, beginning at 1 A.M.

reasons why the thresholds cannot be accurately determined by this method and partly on account of practical difficulties, I confined my experiments to a few notes of the middle range. When I remind you that I often had on hand from fifty to seventy-five moths, you will readily see that it was impossible to test each moth, each time, with the entire range of pitches.

The results of these investigations were recorded upon blanks that were prepared especially for this work. A portion of one of those blanks is reproduced in the preceding table.

After the work on all of the moths had been completed, the contents of these blanks were condensed into the following tables.

## TABLE II.

REACTIONS OF GIANT SILK-WORM MOTHS TO SOUNDS.

| Name of the Specimen. | Number of Individ. | Number of Trials. | Per Cent. of Responses. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 1 to 9. | 10 to 19. | 20 to 29. | 30 to 39. | 40 to 49. | 50 to 59. | 60 to 69. | 70 to 79. | 80 to 89. | 90 to 100. |
| *Samia cecropia* | | | | | | | | | | | | | |
| Males | 38 | 380 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 2 | 0 | 0 | 33 |
| Females | 41 | 615 | 0 | 0 | 0 | 1 | 0 | 1 | 5 | 0 | 1 | 1 | 32 |
| Total | 79 | 995 | 1 | 0 | 0 | 1 | 1 | 1 | 6 | 2 | 1 | 1 | 65 |
| *Philosamia cynthia* | | | | | | | | | | | | | |
| Males | 50 | 950 | 19 | 0 | 0 | 2 | 4 | 4 | 9 | 3 | 1 | 1 | 7 |
| Females | 54 | 875 | 10 | 0 | 1 | 3 | 0 | 1 | 11 | 4 | 4 | 1 | 19 |
| Total | 104 | 1,825 | 29 | 0 | 1 | 5 | 4 | 5 | 20 | 7 | 5 | 2 | 26 |
| *Callosamia promethea* | | | | | | | | | | | | | |
| Males | 23 | 380 | 4 | 0 | 0 | 0 | 3 | 0 | 5 | 0 | 0 | 1 | 10 |
| Females | 18 | 495 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 2 | 5 | 2 | 6 |
| Total | 41 | 875 | 5 | 0 | 0 | 0 | 4 | 0 | 6 | 2 | 5 | 3 | 16 |
| *Telea polyphemus*[1] | | | | | | | | | | | | | |
| Males | 39 | 950 | 36 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Females | 39 | 950 | 39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 78 | 1,900 | 75 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |

[1] The above table does not record the three specimens of *T. polyphemus*, which were used in the special tests recorded on pages 333–334.

## TABLE III.

RESPONSES OF *Samia cecropia* TO SOUND.

| Instrument. | Pitch Vibra. per Second. | Individuals Participating. | | | Number of Trials. | | | Per Cent. of Response. | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Males. | Females. | Total. | Males. | Females. | Total. | Males. | Females. | Total. |
| O.P. | 64 | 1 | 4 | 5 | 5 | 35 | 40 | 100 | 100 | 100 |
| O.P. | 128 | 2 | 1 | 3 | 10 | 5 | 15 | 50 | 100 | 67 |
| O.P. | 256 | 6 | 13 | 19 | 25 | 75 | 100 | 100 | 100 | 100 |
| O.P. | 512 | 1 | 2 | 3 | 5 | 15 | 20 | 100 | 100 | 100 |
| P.P. | 680 | 26 | 34 | 60 | 310 | 360 | 670 | 94 | 89 | 91 |
| P.P. | 870 | 2 | 2 | 4 | 15 | 10 | 25 | 33 | 0 | 20 |
| G.W. | 3,480 | 11 | 19 | 30 | 60 | 100 | 160 | 100 | 70 | 81 |
| G.W. | 4,645 | 0 | 1 | 1 | 0 | 5 | 5 | | 100 | 100 |
| G.W. | 6,200 | 1 | 0 | 1 | 10 | 0 | 10 | 50 | | 50 |
| G.W. | 6,960 | 0 | 1 | 1 | 0 | 5 | 5 | | 100 | 100 |
| G.W. | 9,290 | 1 | 0 | 1 | 5 | 0 | 5 | 100 | | 100 |

Explanation of abbreviations used in above table: O.P., organ pipe; P.P., pitch pipe; G.W., Galton whistle (Edlemann's).

## TABLE IV.

EFFECT OF AGE ON THE RESPONSES OF *S. cecropia* TO SOUND.

| Age in Days. | Individuals Participating. | | | Number of Trials. | | | Per Cent. of Responses. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Males. | Females. | Total. | Males. | Females. | Total. | Males. | Females. | Total. |
| 0–1 | 26 | 23 | 49 | 205 | 200 | 405 | 80 | 80 | 80 |
| 1–2 | 7 | 10 | 17 | 60 | 55 | 115 | 100 | 82 | 91 |
| 2–3 | 5 | 11 | 16 | 30 | 65 | 95 | 100 | 54 | 72 |
| 3–4 | 2 | 12 | 17 | 10 | 120 | 130 | 100 | 100 | 100 |
| 4–5 | 4 | 4 | 8 | 20 | 25 | 45 | 100 | 100 | 100 |
| 5–6 | 2 | 3 | 5 | 10 | 20 | 30 | 100 | 75 | 83 |
| 6–7 | 4 | 3 | 7 | 25 | 30 | 55 | 100 | 83 | 91 |
| 7–8 | 1 | 2 | 3 | 15 | 20 | 35 | 100 | 100 | 100 |
| 8–9 | 1 | 2 | 3 | 10 | 15 | 25 | 100 | 100 | 100 |
| 9–10 | 2 | 4 | 6 | 25 | 30 | 55 | 100 | 100 | 100 |
| 10–11 | 0 | 3 | 3 | 0 | 15 | 15 | | 100 | 100 |

## TABLE V.

EFFECT OF TEMPERATURE ON THE RESPONSES OF *S. cecropia* TO SOUNDS.

| Temperature in F. Degrees. | Individuals Participating. | | | Number of Trials. | | | Per Cent. of Responses. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Males. | Females. | Total. | Males. | Females. | Total. | Males. | Females. | Total. |
| 50–59 | 11 | 6 | 17 | 130 | 70 | 200 | 83 | 53 | 70 |
| 60–69 | 9 | 14 | 23 | 130 | 110 | 240 | 74 | 87 | 80 |
| 70–79 | 4 | 16 | 20 | 40 | 170 | 210 | 100 | 94 | 95 |
| 80–89 | 18 | 27 | 45 | 155 | 235 | 290 | 97 | 96 | 96 |
| 90–99 | 0 | 4 | 4 | 0 | 35 | 35 | | 100 | 100 |

## TABLE VI.

EFFECT OF MATING ON THE RESPONSES OF *S. cecropia* TO SOUND.

| | Number of Individuals. | Number of Trials. | Per Cent. of Responses. |
|---|---|---|---|
| Males: | | | |
| Unmated..... | 31 | 320 | 88 |
| Mated ..... | 7 | 120 | 97 |
| Total..... | 38 | 440 | 90 |
| Females: | | | |
| Unmated..... | 36 | 520 | 86 |
| Mated..... | 5 | 55 | 73 |
| Total..... | 41 | 575 | 85 |
| Grand total..... | 79 | 1,015 | 88 |

## TABLE VII.

RESPONSES OF *Philosamia cynthia* TO SOUND.

| Temperature in F. Degrees. | Individuals Participating. | | | Number of Trials. | | | Per Cent. of Responses. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Males. | Females. | Total. | Males. | Females. | Total. | Males. | Females. | Total. |
| 60– 69 | 25 | 23 | 48 | 145 | 120 | 265 | 31 | 42 | 33 |
| 70– 79 | 43 | 43 | 86 | 410 | 420 | 830 | 36 | 60 | 48 |
| 80– 89 | 23 | 34 | 57 | 245 | 300 | 545 | 33 | 67 | 51 |
| 90– 99 | 6 | 11 | 17 | 130 | 65 | 195 | 58 | 77 | 67 |
| 100–109 | 0 | 8 | 8 | 0 | 40 | 40 | | 88 | 88 |

## TABLE VIII.

EFFECTS OF MATING ON THE RESPONSES OF *Philosamia cynthia* TO SOUND.

| | Number of Individuals. | Number of Trials. | Per Cent. of Responses. |
|---|---|---|---|
| Males: | | | |
| Unmated..... | 47 | 880 | 38 |
| Mated..... | 8 | 65 | 31 |
| Total..... | 55 | 945 | 36 |
| Females: | | | |
| Unmated..... | 50 | 835 | 63 |
| Mated..... | 8 | 90 | 56 |
| Total..... | 58 | 925 | 61 |

## Table IX.

Effect of Age on the Responses of *Philosamia cynthia* to Sound.

| Age in Days. | Individuals Participating. | | | Number of Trials. | | | Per Cent. of Responses. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Males. | Females. | Total. | Males. | Females. | Total. | Males. | Females. | Total. |
| 0–1 | 45 | 51 | 96 | 645 | 400 | 1,045 | 34 | 58 | 43 |
| 1–2 | 26 | 19 | 45 | 290 | 160 | 450 | 49 | 68 | 57 |
| 2–3 | 12 | 18 | 30 | 160 | 365 | 525 | 38 | 73 | 53 |
| 3–4 | 19 | 19 | 38 | 145 | 105 | 250 | 32 | 58 | 42 |
| 4–5 | 6 | 12 | 18 | 45 | 70 | 115 | 32 | 64 | 48 |
| 5–6 | 1 | 6 | 7 | 5 | 30 | 35 | 100 | 83 | 85 |
| 6–7 | 1 | 1 | 2 | 5 | 5 | 10 | 0 | 100 | 50 |
| 7–8 | 2 | 4 | 6 | 10 | 20 | 30 | 0 | 50 | 34 |
| 9–10 | 0 | 2 | 2 | 0 | 10 | 10 | 0 | 50 | 50 |

## Table X.

Effect of Temperature on the Responses of *Callosamia promethea* to Sound.

| Temperature in F. Degrees. | Individuals Participating. | | | Number of Trials. | | | Per Cent. of Responses. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Males. | Females. | Total. | Males. | Females. | Total. | Males. | Females. | Total. |
| 50–59 | 1 | 0 | 1 | 5 | 0 | 5 | 100 | | 100 |
| 60–69 | 7 | 3 | 10 | 50 | 20 | 70 | 70 | 75 | 71 |
| 70–79 | 10 | 13 | 23 | 125 | 125 | 250 | 72 | 80 | 76 |
| 80–89 | 16 | 18 | 34 | 135 | 250 | 385 | 56 | 76 | 69 |
| 90–99 | 10 | 18 | 28 | 70 | 150 | 220 | 71 | 87 | 82 |
| 100–109 | 0 | 8 | 8 | 0 | 40 | 40 | | 63 | 63 |

## Table XI.

Effect of Mating on the Responses of *Callosamia promethea* to Sound.

| | Number of Individuals. | Number of Trials. | Per Cent. of Responses. |
|---|---|---|---|
| Males: | | | |
| Unmated..... | 19 | 375 | 63 |
| Mated..... | 3 | 55 | 82 |
| Total..... | 21 | 430 | 67 |
| Females: | | | |
| Unmated..... | 15 | 515 | 80 |
| Mated..... | 3 | 62 | 80 |
| Total..... | 18 | 575 | 80 |

A careful perusal of the tables I–XII. shows that *S. cecropia*, *P. cynthia* and *C. promethea*, respond to a long range of sound waves. Since precautions were taken to prevent vibrations reaching them through any medium other than air, it seems

## Table XII.

Effect of Age on the Responses of *Callosamia promethea* to Sound.

| Age in Days. | Individuals Participating. | | | Number of Trials. | | | Per Cent. of Responses | | |
|---|---|---|---|---|---|---|---|---|---|
| | Males. | Females. | Total | Males. | Females. | Total. | Males. | Females. | Total. |
| 0–1 | 17 | 13 | 30 | 155 | 105 | 260 | 74 | 76 | 75 |
| 1–0 | 14 | 14 | 28 | 125 | 135 | 260 | 68 | 89 | 79 |
| 2–3 | 11 | 13 | 24 | 60 | 125 | 185 | 50 | 92 | 78 |
| 3–4 | 7 | 10 | 17 | 35 | 75 | 110 | 30 | 75 | 58 |
| 4–5 | 2 | 8 | 10 | 10 | 105 | 115 | 100 | 57 | 61 |
| 5–6 | 1 | 6 | 7 | 5 | 45 | 50 | 0 | 56 | 50 |
| 6–7 | 0 | 1 | 1 | 0 | 5 | 5 | | 100 | 100 |
| 7–8 | 0 | 1 | 1 | 0 | 5 | 5 | | 100 | 100 |

reasonable to conclude that they hear. How about *Telea polyphemus?* Of the seventy-eight individuals whose behavior is recorded in Table II. only three made any responses whatever. Of these three, two gave over ninety per cent. of responses and the other less than thirty. Shall we conclude that *Telea polyphemus* is deaf and that these few responses were due to some factor overlooked by the investigator; or, shall we consider the responses made by all of these moths as expressions of emotion, and attribute the non-responsiveness of *polyphemus* to a sluggish temperament?

To one who has worked much with *Telea polyphemus*, this last suggestion is fascinating; for this moth is exceptionally unresponsive to all ordinary stimuli. The opposite sex is about the only thing that arouses much activity. There is another possibility. *Telea polyphemus* is not a very conspicuous object; indeed, in certain situations, it might be considered protectively colored. It may be that correlated with this inconspicuous coloration is an instinct to remain rigidly immobile in the presence of all ordinary stimuli. To test the matter the following experiments were conducted.

A freshly emerged *Telea polyphemus*, the wings of which had become thoroughly dry, was tested with an organ pipe set to produce 256 vibrations per second. As was to be expected, there was no visible response. The organ pipe was then sounded five times in rapid succession. Immediately thereafter, the insect was roughly handled for a few minutes. It was tossed

about, gently squeezed and thrown upon its back. This was repeated over and over again, sometimes in one order and sometimes in another. After the moth had quieted down, the pipe was sounded five times in rapid succession. Each time the pipe was sounded, the moth waved its wings vigorously. At intervals of two hours, this experiment was repeated from early morning until dark. Invariably the moth responded in the same manner. On the following day the experiment was continued with the same moth. The result was always the same.

About a week later, similar experiments were conducted with two other specimens of the same moth. These, like the one used above, were females. With two exceptions, the results were identical. The exceptions were as follows: (1) one of the moths instead of moving its wings vigorously moved them slowly; the other two moths moved their wings so vigorously that they were lifted off of the support; in this case the body remained on the support, although the wings moved each time the whistle blew; (2) on two occasions a moth that had been experimented upon several times, instead of waiting for the five tones that were produced after the handling, waved its wings vigorously to each of the five preliminary notes. Evidently *Telea polyphemus* can hear. These experiments induced in those moths a state of nervous excitability which caused them to respond to the sounds produced.

## Conclusions.

1. It seems certain that all four of the species of giant silkworm moths investigated can hear. Three of the species respond readily to a large range of sounds. The third, *Telea polyphemus*, normally does not respond to sounds; unless remaining as immobile as possible be considered a response. By experimentally causing the moth to associate some disagreeable experience with certain sounds, it can be induced to respond to those sounds.

2. There is much evidence that the responses of moths to stimuli are expressions of emotion. The fact that an insect does not respond to a sound is no sign that it does not hear it. The response depends upon whether or no the sound has a life significance.

# A PRELIMINARY ACCOUNT OF SOME CYTOLOGICAL CHANGES ACCOMPANYING DESICCATION.

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The ability of certain rotifers, tardigrades and nematode worms to withstand periods of desiccation has been a subject of investigation for many biologists throughout a period of more than two hundred years. Beginning with von Leeuwenhoek in 1701 and extending to the present time, researches have been carried on at intervals, in the case of the Bdelloid rotifers, with the object of determining whether or not these animals can undergo a true desiccation. The results of the several authors have shown a striking variance, in fact in some cases the conclusions of one worker have been directly opposed to those of some other who used the same species of animal in his experiments. The latest publication upon this subject is that of Jacobs ('09) who worked on the Bdelloid rotifer, *Philodina roseola*. He concludes, after prolonged experimentation and as a result of chemical and physical tests as well as by other indirect methods, that this animal undergoes a true desiccation; that at all times the cuticle is freely permeable to water and gases and that no evidence of a waterproof cyst can be found. He notes further that desiccation is usually followed by a period of reproductive activity. The foregoing conclusions together with others not bearing directly upon the subject of this paper have been confirmed during the course of this study.

Jacobs, while conducting his investigation in a very thorough manner, made no attempt to determine, from a histological or cytological point of view, the condition of the tissues of the desiccated animals as compared with those in the normal individual. To the knowledge of the author no comparison of this sort has been attempted by any investigator up to the present time. At the suggestion of Dr. E. G. Conklin, I have undertaken an inquiry into this last question and present here a brief account

of the results obtained to date. A more detailed account based upon further work will appear in a future publication.

The anatomy of the Philodinidæ has been described by Janson (1893). The most conspicuous organs in the living animal are those of the reproductive and the alimentary systems. The latter begins with the buccal cavity at the base of the trochal organs. This narrows down to a short tube leading to the mastax. The mastax, with the digestive glands surrounding it, is followed by the thin-walled esophagus which leads into the thick-walled stomach. The posterior end of the latter is continued by the "*blasendarm*" which leads to the cloaca and thence to the anus. The reproductive organs consist of two more or less spindle-shaped bodies lying on either side of the stomach. These are the ovaries and the vitellaria and they may at all times be recognized by their prominent nuclei. The nephridia with their flame cells are easily observed in the living animal. In the foot are found the glands which secrete a substance which enables the animal to adhere to different objects. The head region contains several large coronal cells with large nuclei.

A cross section of a normal extended *P. roseola* through the mid-body region is shown in Fig. 1. The vitellaria have enormous nuclei, consisting of a central karyosome surrounded by a clear homogeneous area and peripherally by a distinct nuclear membrane. This is the "nucleolar nucleus" of Carnoy and this type of nucleus is characteristic of the greater part of the cells of the animal. The cytoplasm of the vitellarium, which is syncytial in nature, is made up of granules of varying sizes and these granules appear in different patterns particularly when a variety of fixatives are used; it has quite an affinity for nuclear stains as is usual with yolk structures. The cytoplasm of the ovary does not differ essentially from that of the vitellarium. Ovary and vitellarium are each surrounded by a thin membrane. Figs. 1 and 4 show characteristic sections through normal reproductive glands.

The cytological structure of the stomach of the Philodinidæ has been described by Zelinka ('86) in the case of *Callidina symbiotica*, by Janson ('93), and more recently by de Beauchamp ('09), in the case of *Callidina socialis*. The lumen, the position

of which in the stomach tissue is not constant, is lined with a heavy ciliated cuticula. Just beneath the cuticula are found longitudinal muscle fibers arranged at regular intervals. The part of the stomach outside the thin layer of muscle fibers is syncitial in nature. With the iron-hæmatoxylin-eosin-lichtgrün stain of de Beauchamp three elements may be distinguished, (1) nuclei having, in general, the same structure as those described for the vitellarium; (2) densely staining granules of great size, not surrounded by a clear area or membrane. These are probably aggregations of food material. (3) Vacuoles of greater or less size which stain with lichtgrün. These last are probably globules of excretory material as de Beauchamp has pointed out. In the latter's description of the stomach of *Callidina socialis* he says that the stomach is not surrounded by an "individualized membrane" but only by a layer of protoplasm which projects at the periphery. In *P. roseola*, as far as I have been able to determine, a true membrane is present (Fig. 1).

The skin of *P. roseola* has practically the same structure as that of other rotifers. It consists of two layers, cuticula and hypodermis. The former is the more densely staining layer and is composed of fine granules closely packed together; the latter is a finely reticulated plasma layer in which cell boundaries cannot be distinguished and in which nuclei are found scattered at irregular intervals. The skin is pliable and may be readily folded at any point. It is difficult to obtain sections in which one or more of these folds do not appear.

The brain of *P. roseola* is of an elongated triangular shape and lies in front of and slightly above the mastax. Zelinka ('88), in the case of *Discopus synaptæ*, has figured the brain as a syncitium in which the nuclei are closely packed together about the periphery, while in the central part is found the "*punkstubstanz*," a finely granular portion without nuclei. In the greater number of cases I have been able to distinguish definite cell boundaries in the case of the cells forming the peripheral layer of the brain of *P. roseola*. The nuclei of these cells are uniformly circular in section and contain a small amount of chromatin scattered in irregular masses through a homogeneous nuclear plasm. The cytoplasm is homogeneous and has the appearance of a colorless

fluid. The "*punktsubstanz*" lies approximately at the center of the organ and is granular in structure.

In the fully extended living *Philodina* the ciliated or trochal discs are prominent at the anterior end of the body. The cilia upon these discs by their successive beating give the effect of a revolving wheel. When the animal is disturbed the discs are folded and retracted into the pharyngeal region where they may be observed as oval patches. The alimentary canal is also ciliated throughout almost its entire length.

Of the glandular structures, other than those employed for reproduction, the slime glands of the foot are perhaps most easily seen. These consist of rows of cells whose cytoplasm is alveolar or finely reticular. The nuclei are large and may sometimes be seen in the living animal. The digestive glands in the region of the mastax are similar to the foot glands in structure and staining qualities.

The changes in cell organization which accompany the process of desiccation are fairly uniform in result for all the tissues. Although slight variations have been observed, these are differences of degree and not of kind. Since the cellular elements are larger and hence more easy to observe in the vitellarium, this organ will be considered first.

In a section of the vitellarium of a desiccated *P. roseola* the most noticeable difference from the conditions which are present in the normal tissues are seen in the nucleus. Normally, as was stated before, the nuclear membrane, though definite, is not very thick. Just within the membrane is a ring of homogeneous ground substance or nuclear sap. In the center of the nucleus is found the large, densely staining karyosome. In the dried animal these conditions are exactly reversed. The karyosome may disappear entirely but if this extreme condition does not come about, the structure which remains in the position of the karyosome is similar neither in shape nor in staining qualities to the original element. In extreme cases the central area of the nucleus in the dried organ has exactly the same appearance as the clear area of the normal nucleus. The nuclear membrane becomes heavy and has the appearance of a thick ring (Fig. 5). In most cases it appears to be composed of fine granules closely

packed together. Under conditions mentioned hereafter this granular appearance may give place to a dense homogeneous black ring (iron-hæmatoxylin preparations) staining exactly like the normal karyosome. The changes in the cytoplasm, though distinct, are much less marked than the nuclear changes. With the withdrawal of water the cytoplasm increases in density and loses the regular arrangement of its particles which is characteristic of the normal vitellarium (Fig. 4). The yolk granules become arranged irregularly or in small closely packed groups as in Fig. 5. The drying process causes a loss of staining power in the tissue.

The shrinkage of the cytoplasmic portion of the tissues is well demonstrated in the case of the hypodermis. Fig. 1 show as section of this layer of the skin as it appears in the animal living under normal conditions. In a section through the dried animal (Fig. 2) it will be noticed that the hypodermal layer has shrunken markedly, approaching its normal thickness only in those places where the nuclei are located. The nuclei apparently do not diminish in size and they cause a protuberance in the dried hypodermis wherever they are found. The nuclear material is redistributed in the same manner as was described for the vitellarium.

This arrangement of the nuclear elements is found in practically all the tissues of the dried animals. A detailed description of the changes in the other organs would be, for the most part, mere repetition.

As was mentioned above, the cilia in *P. roseola* are well developed, both in the head region and in the digestive tract. It would seem that a fiber of such delicate texture as that of a cilium would not long survive the effects of a removal of moisture. Such, however, is not the case. Not only do the trochal cilia escape serious injury by the desiccation process but the same is also true of those in the digestive canal. Fig. 2 shows a section cut through the infolded trochal discs of a dried animal. There is no sign of any fusion or other abnormal condition of these elements. Each cilium preserves its identity apparently as well as would those of an animal living in a natural environment.

The changes in cell structure attending recovery from desicca-

tion are almost the exact opposite of those just described. In cases where the karyosome has entirely disappeared it begins to form again in its proper position a short time after water is added to the dried animals. The thickened nuclear membrane described above shows a greater affinity for stains at this stage and gradually assumes its normal thickness. Cytoplasmic changes are quite noticeable at this time. In the vitellarium (Fig. 6) it is frequently noticed that the material surrounding the nucleus is aggregated into strands or other irregular patterns. This would seem to indicate that the cytoplasm is more freely permeable to water in certain regions than in others and that the stage represented in Fig. 7 shows a step in the gradual redistribution of extranuclear substance attending recovery from desiccation. In the case of the other organs, as before, the process of recovery is very similar. The elements are much smaller in some cases and hence more difficult to observe but the mechanism as well as the result seems to be the same.

It has been suggested to the author that the cytoplasmic and the nuclear changes taking place in dry seeds might be analogous to the ones in the rotifers just described. With this in mind, sections of the embryo of the common Indian corn, *Zea mais*, were cut, (1) at the time the seeds were fully ripened but had not become entirely dried; (2) after the seeds were thoroughly dried; and (3) after the seeds were well germinated. A section of a typical procambium cell from each of these stages is shown herewith. Fig. 14 shows a cell from a germinating embryo. It will be noticed that the cytoplasm contains many spaces filled with cell sap. The nucleus has a ring of chromatic material just within the nuclear membrane. The nucleolus is vacuolated and does not stain in the same manner as the chromatic ring at the periphery of the nucleus. The nucleolus is surrounded by a clear area which probably consists of fluid material. Fig. 15 shows the conditions which exist when the embryo is partially dried. The chromatic ring thickens, diminishing the space between it and the nucleolus. The latter becomes more compact and the vacuoles disappear. An extreme case of drying is shown in Fig. 16. The cytoplasmic granules are closely and regularly packed together. The clear space in the nucleus has disappeared

and the substance of the nucleolus has apparently diffused throughout the nuclear area.

The changes described for the drying corn cells in the last paragraph are at first sight remarkably like those occurring in the rotifer during desiccation. In both rotifer and corn the nucleus contains a nucleolus surrounded by a clear space, while around the two is a chromatic membrane of varying thickness. When water is removed the clear space around the nucleolus disappears and comes into existence again only upon the addition of water. The substance of the nucleolus in both cases diffuses toward the periphery of the nucleus leaving a more or less clear space in the center of the same. In the cytoplasm also there is a parallel between the behavior of the cells of the two forms. Loss of water is attended by shrinkage and a consequent increase in density. The cytoplasmic materials tend to gather in small lumps which remain closely packed together until moisture is again applied.

Whether the seemingly similar processes in these representatives of the plant and animal kingdom are indeed analogous can be determined only after further study.

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## Description of Plate I.

Fig. 1. Cross section through the mid-body region of a normal expanded *Philodina roseola*. Leitz compensating ocular 4, obj. 2mm.

Fig. 2. Section of a rotifer kept for eighteen days previous to fixation in an evacuated calcium chloride desiccator. Leitz oc. 4, obj. 2mm.

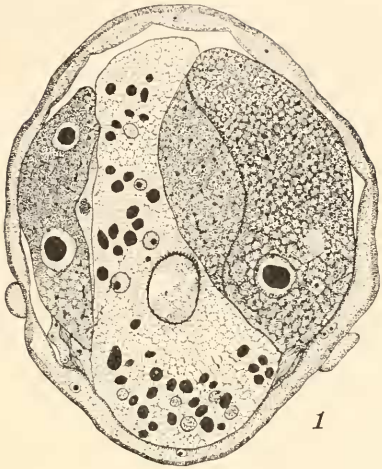
Fig. 3. Section through a normal animal, not expanded. Leitz oc. 4, obj. 2mm.

Fig. 4. Section through the vitellarium of a normal animal. Leitz oc. 12, obj. 2mm.

Fig. 5. Section of the vitellarium of an animal dried in an evacuated desiccator for fourteen days previous to fixation. Leitz oc. 8, obj. 2mm.

Fig. 6. Longitudinal section of an animal recovering from desiccation. The rotifer was kept in an evacuated desiccator for fifteen days, then placed in water for an hour and fifteen minutes, at the end of which time it was fixed. Leitz oc. 8, obj. 2mm.

Fig. 7. Cross section of vitellarium of animal recovering from desiccation. Animal was kept in an evacuated desiccator for six days, then placed in water for one hour, at the end of which time it was fixed. Leitz oc. 8, obj. 2mm.



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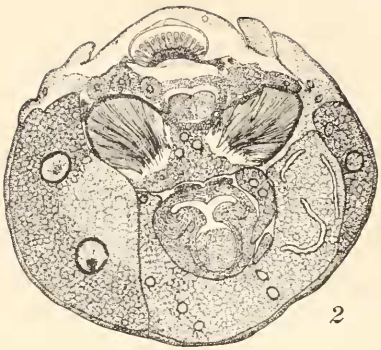

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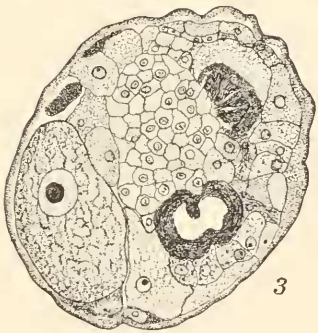

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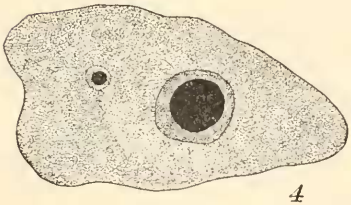

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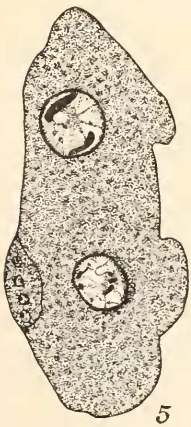

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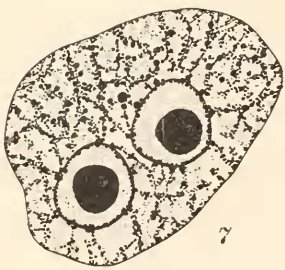

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PLATE II.

FIG. 8. Section of brain of *P. roseola*. Normal active animal. Leitz oc. 8, obj. 2mm.

FIG. 9. Section of brain of a rotifer which was kept in an evacuated desiccator for fourteen days previous to the time of fixation. Leitz oc. 8, obj. 2mm.

FIG. 10. Section of brain of a rotifer which was dried twenty-four hours, put in water for one hour and then killed. Leitz oc. 8, obj. 2mm.

FIG. 11. Section of foot gland cells from a normal active animal. Leitz oc. 12, obj. 2 mm.

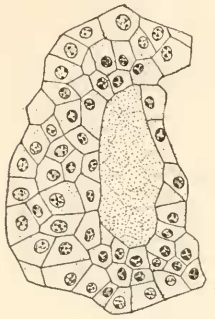
FIG. 12. Section of foot gland ells of a rotifer which was kept in an evacuated desiccator for fourteen days previous to the time of fixation. Leitz oc. 12, obj. 2mm.

FIG. 13. Section of foot gland cells from an animal kept fourteen days in an evacuated desiccator and then placed in water for one and one fourth hours previous to fixation. Leitz oc. 12, obj. 2 mm.

FIG. 14. Section of procambium cell from a germinating corn embryo. Leitz oc. 8, obj. 2mm.

FIG. 15. Section of procambium cell from a partially dried corn embryo. Leitz oc. 8, obj. 2mm.

FIG. 16. Section of procambium cell from a corn embryo dried for a month at room temperature. Leitz oc. 8, obj. 2 mm.



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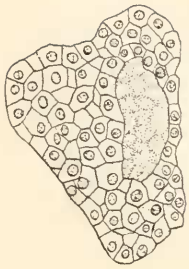

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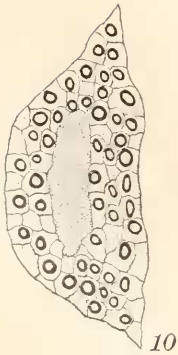

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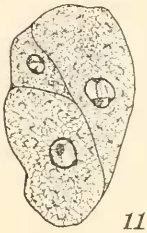

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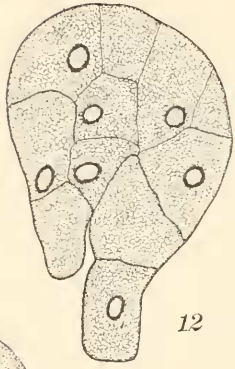

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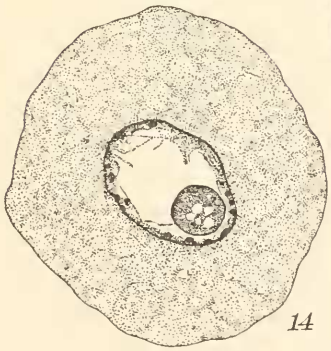

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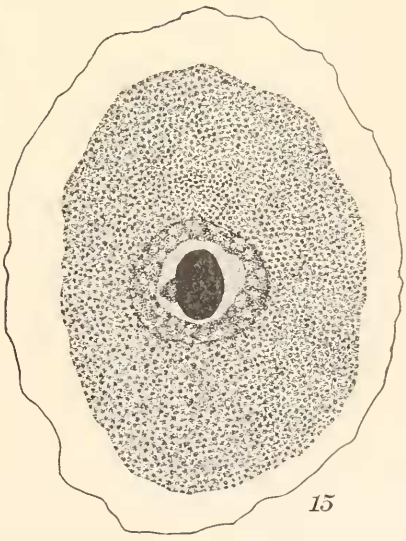

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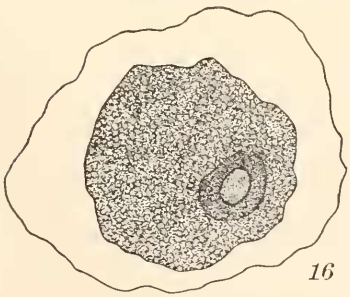

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# REGULATION IN VORTICELLA.

E. M. RUNYAN AND H. B. TORREY.

(From the Department of Biology, Reed College, Portland, Oregon.)

It is a fact well known to students of regeneration that one part of an organism may exert a measurable influence over the growth and development of another. This has been demonstrated for many of the Metazoa under varying forms. The removal of the head of a planarian liberates, as it were, the postjacent tissue, out of which a new head is fashioned. Among macruran crustaceans, the loss of the larger chela of an asymmetrical pair has been shown many times to be succeeded by an accelerated growth of the smaller chela and a subsequent retardation in the regeneration of the lost chela so that, in the presence of the small chela grown large, it remains the smaller of the two. Finally—not to multiply instances needlessly—when a short length of the column, with hydranth, is cut away from the hydroid *Corymorpha*, no development beyond closure of the wound occurs proximally until the hydranth is removed from the distal end. In this respect, the behavior of *Corymorpha* may be contrasted with the behavior of the planarian, since in the latter the presence of the original head on the anterior piece does not inhibit the development of a tail posteriorly. The hydranth in *Corymorpha* appears somehow to inhibit, in short pieces, even the development normally to be expected at the aboral end.

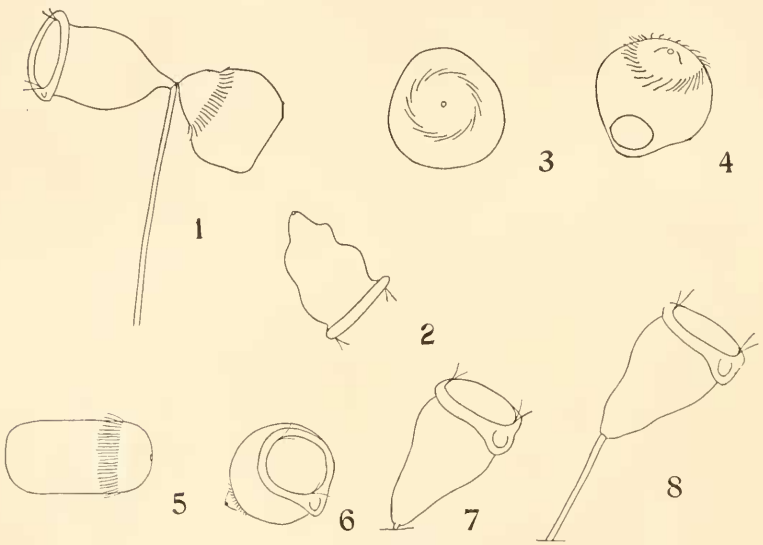
Among the Protozoa, instances of this last sort seem so generally to have escaped record, that we have thought it desirable to describe a similar domination in *Vorticella* sp. of one part over another.

When *Vorticella* divides, the fission plane passes approximately through the center of the organism from oral disk to a point immediately to one side of the contractile stalk. Of the zoöids thus formed, one remains attached to the original stalk, while the other swims away by means of cilia which, during the

last phases of the process of fission, have appeared in a circlet near the aboral pole (Fig. 1).[1]

Why do such cilia not appear on the stalked zoöid?

It may be noticed in the last stages of fission that the zoöid destined to become free retains its connection by a slender protoplasmic strand with the body of the stalked zoöid, not directly with the stalk itself. This fact suggests what has proven to be the correct view, namely, that cilia which would normally develop on every individual are able to show themselves only when sufficiently isolated physiologically from the stalk. Such isolation exists when the connection between the separating zoöids is reduced to a narrow strand.




FIGS. 1–8.

This view was reinforced by the familiar fact that, upon becoming attached to the substrate, the free zoöid gradually loses its cilia as its stalk develops. In the normal life history, then, aboral cilia develop in isolation from the stalk and disappear with the development of the stalk.

The test was applied by cutting a stalked zoöid quite away from the stalk. This was accomplished under a binocular, by

[1] The figures have not been drawn with a camera. Their scale varies somewhat.

means of a sharp dissecting needle. A typical case is shown in Figs. 2–8. Soon after the cutting, the zoöid (Fig. 2) settled down on its oral surface. In an hour, cilia began to push out in a circlet near the aboral pole. They elongated rapidly, and began to beat around the oral-aboral axis (Fig. 3). The oral disk turned in upon itself in the manner characteristic of the normally free zoöid (Fig. 4). One hundred and five minutes after the operation, the zoöid swam away (Fig. 5), indistinguishable in every respect from the normally free form. After five minutes of active locomotion, it came to rest on its aboral end, became attached, and unfolded its oral disk (Fig. 6). At once the stalk began to grow and the aboral cilia to disappear. In ten minutes no aboral cilia were to be seen (Fig. 7). Two hours and a half later, the organism appeared as in Fig. 8.

The development of the stalk appears to be dependent on contact at the aboral end; while the development of aboral cilia is conditioned by physiological isolation from the stalk whether achieved experimentally or by a narrowing of protoplasmic connection in the ordinary course of fission.